Exploring genetics associated with phenology and grain quality in barley (Hordeum vulgare L.)

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Declaration of originality

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Publications

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Statement of co-authorship

The thesis was completed during the course of my enrolment in a PhD degree in the School of Land and Food at The University of Tasmania. The thesis contains no experimental results that have previously presented for any degree at this or other institutions.

The thesis contains one literature review chapter and three major research chapters. The literature review (Chapter 2) has been published as a book chapter. Results described in the three research chapters have been or will be published in different journals.

The following people and institutions contributed to the published work undertaken as part of the thesis:

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Abstract

Barley (*Hordeum vulgare*) is ranked fourth among the cereals in the world today and second in size to wheat in Australia. It is an important crop mainly used for malting and feed. Climatic extremes and variability often limit potential barley production. Frost and drought, for example, may result in up to 80% and total grain yield loss, respectively. The most vulnerable growth period of an actively growing cereal crop is the reproductive phase, including the spikelet initiation, heading and grain filling stages. Heading date is one of the most important traits for improving barley stress tolerance to drought, heat, waterlogging and frost. Well timed duration and date of ear emergence and grain filling can improve yield in environments prone to frost, water deficits and/or heatwaves. Therefore, identification of the genetic factors controlling these traits and mechanisms of regulation including their interactions will facilitate breeding efforts to adapt genotypes for specific environment given the challenges of climate variability facing barley production.

Although ear emergence in barley is a quantitative trait that is influenced by vernalisation, growing-degree-days (*GDD*) and daylength, the trait is primarily controlled by three groups of genes. Genes with major effects include photoperiod, *Ppd* (*Ppd-H1* and *Ppd-H2*) on chromosomes 2H and 1H respectively, and vernalisation, *Vrn* (*Vrn-H1, Vrn-H2, Vrn-H3* and *Vrn-H4*), on chromosomes 5H, 4H, 7H and 5H, respectively. Genes with minor effects include *earliness per se Eps* and *HvPHYC*. The *Ppd*, *Vrn* and *Eps* genes have been deployed in many breeding programs so that ear emergence and grain-filling periods may occur within favourable conditions, and with consequent stress avoidance thereby optimising biomass production and yield potential.

Wild barley genotypes show a diversity in various traits from cultivated barley. Using a doubled haploid barley population sown in three seasons differing in daylength and temperature, quantitative trait loci controlling heading date from different populations and growing seasons were investigated. Of the two parents, SYR01 is a Syrian wild barley and Gairdner is an Australian malting barley. A total of 173 DH lines were genotyped with DArT and SNP makers. The heading dates were phenotyped using three sowing dates (winter and spring and summer) to test the photoperiod/temperature responses of different QTL. DH lines showed a transgressive segregation; DH lines were significantly different from the parents in heading date. Eight QTL were identified for heading date from different sowing dates under field conditions, accounting for 7-47% of the phenotypic variation. The locations of most QTL in this study coincide with the positions of the earlier reported genes, including Ppd-H1 on 2H, Eps genes on 3H, Vrn-H2 on 4H. Others include Vrn-H1, Eps on 5H, Eps.6S on 6H and Vrn-H3 on 7H. Vrn genes were only identified in spring sowing trials. One QTL (Qhd-sg-2H.1w) on 2H was located in a different position from Ppd-H1 and (Qhd-sg-5HL.3s) on 5H; these QTL requires further investigation to confirm if they are new genes for heading date. This study confirmed the results of previously reported genes, most of which have been conducted in greenhouse. Our study demonstrates the important role of genetic interaction that regulates heading date under natural production environments in barley. Finally, this study showed that the QTL within region of 122.5 and 126.1 cM influencing early maturity in the NILs consist of an Eps gene different from Vrn-H1 and HvPHYC and has pleiotropic effects on agronomic traits like spike length and number of spikelets per spike. Cultivation of the early maturing genotype of the NILs is more suitable in drought prone short seasoned environment while the late genotype is more adapted to longer season conditions indicating that process-based models can effectively complement breeding efforts in determining the performance of new

genotypes in new environments under diverse management conditions. The study involving DH lines of SYR01 x Gairdner under field conditions validates the previously identified QTL, which were mostly detected under controlled environment. with two new QTL (*Qhd-sg-2H.1w*) on 2H and(*Qhd-sg-5HL.3s*) located in a different position on 2H and 5H respectively further investigation is required to confirm if they are new genes for heading date with the anticipation of delivering the closely linked molecular markers with these genes to breeders and farmers.

In our previous studies, a new allele for early flowering was identified from the cross between an Australian malting barley cultivar (Franklin) and a Chinese landrace (TX9425). Four sets of near isogenic lines (NILs, *Eps5H-116, Eps5H-317-1, Eps5H-317-2 and Eps5H-322*) were located on chromosome 5H at the interval of 122.0-129.0 cM. Further experiments were conducted to investigate how this gene was regulated by photoperiod using the NILs with three sowing dates from autumn to summer. The NILs carrying the earliness allele were significantly earlier than the late genotype at all sowing dates. This gene was different from previously reported vernalisation genes that are located at a similar position, as no vernalisation was required for the NILs. The difference between this gene and *Eam5* (*HvPHYC*) locus which also located between two co-segregated markers (3398516S5, 122.5 cM, and 4014046D5, 126.1 cM), is that with the existence of *Ppd-H1* (*Eam1*), *Eam5* has no effect on ear emergence under long days while the gene from TX9425 reduced the time to ear emergence. The locus showed no pleiotropic effects on grain pasting properties and agronomic traits except for spike length and number of spikelets per spike, and thus can be effectively used in breeding programs for heading date. The array of early heading dates caused by interactions between *Eam5* gene

from *Eps5H-317-1-E* and other maturity genes provides an opportunity to fine tune heading dates with environment types, which can be critical factor in barley breeding.

Two of the four NILs (*Eps-317-1-E*, and *Eps-317-1-L*) were selected to be used in field experiments which were conducted in Tasmania, Australia, using three sowing dates per year during 2015, 2016 and 2017 to parameterise and test the barley module of the APSIM model (APSIM-Barley). Parental lines Franklin and TX9425 were also parameterised in the model. A genotype by environment by management (GxExM) analysis was then conducted using ten sites across the Australian wheat-belt, with a range of sowing dates, fertiliser rates and planting densities. The early genotype (*Eps-317-1-E*) performed better in environments prone to terminal drought and heat stress effects. This was due to earlier flowering and a propensity for greater transpiration-use efficiency from growth stage (GS) 50 to 87. The late NIL (*Eps-317-1-L*) generally produced higher yield in long-season environments with high rainfall and cool terminal temperatures. Performance of all genotypes was generally better for May sowings, wherein yields of the two NILs were highest. This better yield performance of the NILs was due to heterosis resulting to more biomass and consequently greater harvest index. Overall, our study showed that *Eps-317-1-E* is more adapted to regions prone to drought and heat stress, while *Eps-317-1-L* is more suited to regions with longer growing seasons. This study demonstrates how process-based models can be used in concert with breeding experiments, providing farmers and breeders with opportunities to examine how new genotypes will perform in new environments under diverse management conditions.

Chapter 1 General Introduction

1.1 Background

Agriculture is highly depended on spatial and seasonal climatic conditions (Gammans et al., 2017). By the end of the century, global crop yield is predicted to decline by 17% for winter barley and 34% percent for spring barley due to heat stress (Bailey and Davis, 2017; Wiebe et al., 2015). On the other hand, population growth will result in a 9% increase in demand, thus creating a serious supply-demand mismatch (Ibrahim et al., 2018b; AgResource, 2018). Limited yield gains have been reported in most barley producing countries in Europe (Dawson et al., 2015) and in Australia, where the average yield is as low as 2.0 Mt/ha (Australia, 2015) and, in the latter case, can mostly be attributed to the transient, unpredictable and varying climate (Index Mundi, 2015; Meinke et al., 1993). For example, barley yield loss due to drought and heat stress is about 49-57%, amounting around US$1825 million (Tekle and Alemu, 2016). The effects of drought and heat stress include stunting, leaf wilting, pollen sterility and abortion of fertilised ovules (DPIRD, 2015). Thus, identification of appropriate genotypes with more rapid growth rates can allow early maturation and thus allow crop adaptation to drought and heat stress.

1.2 Environmental control of heading date in barley

Two important environmental drivers of heading date include day length and temperature (Kelleher, 2003; Miralles et al., 2001). In plants, there are three major photoperiod sensitivity classifications: long day, short day and day neutral (Kikuchi and Handa, 2009). Barley falls

under the long day, such that ear emergence will occur earlier in longer days up to a certain threshold, though there are variations in responses among genotypes (Boyd et al., 2008). The photoperiod pathway involves photo-receptors that reset the circadian clock; thereafter, signals are transmitted by florigen via phloem to the apexes (Hill and Li, 2016; Kikuchi and Handa, 2009). The clock receptors of light stimuli are phytochromes (*phyA* to *phyE*) and cryptochromes (*cry1* and *cry2*) which are red and far-red receptors and blue light receptors, respectively (Imaizumi and Kay, 2006).

Temperature is an important factor affecting biological processes in plants. In photoperiod sensitive barley genotypes of Australia, for example, the timing and duration of reproductive stages are rapid under higher temperature in long day lengths, but slower under short day conditions (Hemming et al., 2012). However, for a day neutral genotype the switchover to reproductive development is much slower, despite increases in temperature (15^{o}C to 25^{o}C) and changes in day length (Hemming et al., 2012). Generally, the transition from vegetative to reproductive phases in temperate cereals requires prolonged low temperature (vernalisation) (Cockram et al., 2007).

1.3 Genetic regulation of heading date in barley

Early conventional breeding efforts have indicated genotypic variation in heading date in barley (Boyd et al., 2003; Cuesta-Marcos et al., 2009). Considerable genetic advancement in yield has been achieved using molecular techniques to identify important quantitative trait loci (QTL)/genes regulating ear emergence in barley (Boyd et al., 2003). As a quantitative trait, the QTL/genes regulating heading date in barley are highly responsive to environmental cues such as temperature, vernalisation, growing-degree-days (GDD) and day length (Ibrahim et al., 2016). The groups of the QTL mentioned above are vernalisation, photoperiod and

earliness per se. The first group of the QTL/genes respond to vernalisation, exposure to prolonged low temperature, to accelerate ear emergence (Cockram et al., 2007). They are located on are found on chromosomes 5H (*Vrn-H1* and *Vrn-H4*) (Hill and Li, 2016), 4H (*Vrn-H2*) and 7H (*Vrn-H3*) (Alqudah et al., 2014) The second group of the QTL/genes respond to day length (photoperiod) which are found on chromosomes 2H (*Ppd-H1*) and 1H (*Ppd-H2*) (Alqudah et al., 2014; Laurie et al., 1994). Vernalisation and photoperiod genes have been cloned in past work (Zikhali et al., 2015). The third group consist of *earliness per se* genes, which are found in all the seven chromosomes. Their effects express fully after both vernalisation and photoperiod requirement are met (Laurie et al., 1995; Lewis et al., 2008).

1.3.1 Major photoperiod response genes

Variability of natural photoperiod response in barley is caused by two major genes, *Ppd-H1* and *Ppd-H2* (Alqudah et al., 2014). The dominant allele causing earliness under long days is a pseudo-response regulator gene (*HvPRR37*) called *Ppd-H1*, which is adapted to countries in the Middle East and the recessive mutant (*ppd-H1*) allele has reduced sensitivity to day length and is prevalent in northern Europe (Alqudah et al., 2014; Andrés and Coupland, 2012). The other photoperiod gene *Ppd-H2* responds to short day length which belongs to the *FLOWERING LOCUS T* (*FT*)-like group of genes (Faure et al., 2007), and of the five FT genes, *HvFT3* was named as the candidate (Casao et al., 2011). However, the dominant allele of *Ppd-H2* is well adapted to southern Europe and is being cultivated for early maturity under shorter day length especially when without vernalisation (Casao et al., 2011; Faure et al., 2007) while the recessive allele is a mutant found in winter barley (Faure et al., 2007).

1.3.2 Major vernalisation response genes

Vernalisation is genetically controlled by *Vrn-H1*, *Vrn-H2*, *Vrn-H3* and *Vrn4*. In barley, the *Vrn-H1* (*Sgh2* or *Sh2*) encodes the MADS-box transcription factor similar to *APETA-LA1* gene in *Arabidopsis* (Yan et al., 2003). The dominant allele of this gene is vernalisation insensitive and is responsible for the spring type phenotype (Cockram et al., 2007; Yan et al., 2003) while the recessive allele *vrn-H1* responds to vernalisation, resulting in the winter phenotypes (Yan et al., 2003). Barley also has *Vrn-H2* (*Sgh* or *Sh*) consisting of homologs of CO-like genes (*HvZCCT-Ha, HvZCCT-Hb, and HvZCCT-Hc*) (Karsai et al., 2005). Mutations at the three loci in the recessive alleles are responsible for the spring phenotype (Takahashi and Yasuda, 1971; von Zitzewitz et al., 2005). Another locus is the *Vrn-H3* (*HvFT1*), known as the central floral integrator (*CFI*) affecting the flowering pathways in all temperate cereals including barley (Kikuchi et al., 2009). Similar to other loci, variations at the first intron of this gene are responsible for both winter and spring phenotypes in which the spring allele has higher expression level (Yan et al., 2006). The *Vrn4* was first identified from the wheat cultivar *cv.* Gabo from Australia (Knott, 1959; Yoshida et al., 2010). Unlike the other three *Vrn* which were well characterised, this gene is located on 5H and is not well studied in barley (Yoshida et al., 2010).

1.3.3 Earliness per se genes

Grain yield and yield components are substantially affected by the time and duration of the reproductive stages, primarily from spikelet initiation to grain filling (Alqudah et al., 2014; Ibrahim et al., 2018a). These stages are controlled by factors other than photoperiod and vernalisation genes (Flood and Halloran, 1984). These factors are called *earliness per se* (*Eps*) and have been shown to cause variation in heading date after the requirements for photoperiod and vernalisation response have been fully met by barley genotypes (Lewis et al., 2008). *Eps* genes are said to cause rapid

progression of reproductive stages in cereals leading to early heading and maturity (Lewis et al., 2008). Recently, early heading has been found to have direct and positive relationship with grain yield under dry finishing environment, which has led to increased research on early maturing genotypes (Harris et al., 2018). The effect of early heading genes on agronomic traits such as spike length and number of spikelets (Lewis et al., 2008) grain size and weight (Laurie et al., 1994) have led to increased efforts on the characterisation of the genes. Since earlier identification of the *Eps* on all the seven chromosomes of barley (Laurie et al., 1995), very little progress has been made on their locations and their interactions with environments. In addition, there are few field experiments enabling validation of the available information to explain the interaction of these genes/alleles with the environments (Li, 2018). Unlike other major genes such as *Ppd* and *Vrn*, there are very few reports on the cloning of any *Eps* genes in both barley and wheat (Li et al., 2017) even though candidate genes were reported for these genes, for example, *HvELF3* for *eps1* (Boden et al., 2014), *HvCEN* for *eps2* and *HvLUX/PCL1* for *eps3* (Gawronski et al., 2014). More work is required to understand their effects on agronomic traits and yield components.

1.4 Genotype by environment by management (GxExM) effects

Heading date is controlled by complex interactions of genetic, environmental and management factors (Ibrahim et al., 2018b). Variation in heading date among genotypes due to differences in environment and management in barley (Ibrahim et al., 2018b) calls for more appropriate information on yield performance in the target environment with representative management. GxE analyses have been conducted to understand interactions between genotypes and environments (Hammer et al., 2014). However, higher genetic gain in yield has been attributed to partitioning the effects of management out of GxE approaches (Duvick, 2005; Hammer et al., 2014). Instead of conducting physical experiments in different locations/environments, which is often very expensive and time consuming, breeders can

conduct GxExM analysis using models such as APSIM-Barley (Keating et al., 2003). Li (2018) also suggested the need to quantitatively determine the value of various phenological traits in barley by simulating effects of allele combinations and sowing dates on barley yield across diverse environments by using the crop simulation framework APSIM, which could be of enormous value to breeders and pre-breeders. Thus, matching heading date to a management option within a target environment will assist breeders in design of their breeding programs for selection and adapting cultivars to various environments.

The significance of heading date or growing degree- days to heading (*GDD*) to crop adaptation leading to grain yield and quality cannot be overstated. Reports by Li (2018) have confirmed the lack of information on combinations of the desirable phenology alleles for any specific Australian environments. There is an absence of both field data to validate the adaptability of any reported phenology genes in multiple environments in order to properly understand the inter- and intra-locus interactions as well as with the environment and management to achieve higher yield and quality.

This project aimed to:

1. Map a quantitative trait loci (QTL) regulating early maturity in barley using near isogenic lines.
2. The QTL interact with the environment which is imposed by the sowing dates.
3. To determine the effect of each QTL on grain yield and quality;

4. To parameterise a crop model for barley that describes the interaction of the QTL with environment then conduct a genotype by environment by management (GxExM) analysis for harvest biomass, heading date, grain yield and quality.

5. To identify QTL influencing ear emergence from a wild barley accessions.

Chapter 2 Literature review

Abstract

Barley heading date is important in adapting barley genotypes to different environments. Important factors affecting heading date in barley include growing degree-days, photoperiod and sowing date. Sowing date is a management option that may be used to influence heading date; appropriate sowing time may reduce climatic risks such as frosts and water stress at sensitive periods during crop development. Three major genes control heading date in barley. These genes regulate the influence of photoperiod (*Ppd-H1* and *Ppd-H2*), vernalisation (*Vrn-H1, Vrn-H2 and Vrn-H3*) and the duration of the vegetative phase (*Eps*). The *Ppd-H1* locus on chromosome 2(2H) regulates flowering time under long days. *Ppd-H2* on 2H regulates phenology under short day length. Vernalisation is mainly controlled by three loci (*VRN-H1*, *VRN-H2* and *VRN-H3*), which interact in an epistatic fashion to determine vernalisation sensitivity. Appropriate physiological and simulation frameworks such as that of APSIM-Barley are required to complement breeding efforts to help describe and quantify the effects of environment and management on gene expression and how this genetic expression impacts grain yields and quality in barley.

2.1 Introduction

World barley production was projected to reach 140 million metric tons (MMT) on 50 m hectares at 2016/17 (IGC, 2015) with its greatest economic impact being related to its use as feed, human food and malting. The demand is projected to reach 142 MMT (IGC, 2015) by 2023. Australia's barley production annually is the fourth largest in the world, producing about 8.7 MMT of barley in 2015, contributing up to 30% of the world supply (Barley Australia,

2015). The supplies comprise 2.5 MMT of malting barley (CGIAR, 2015; Saisho et al., 2011). Currently, one-third of the world production is used for malting (Baik et al., 2008). The grain is also widely used for human food and livestock feeds, starch production and chemical industries while the straw could be used for roofing huts and animal bedding. Grazing is sometimes performed after harvesting or when the crop is vegetative (Akar et al., 2004; CGIAR, 2015).

There are constraints facing barley-producing nations such as Australia; including transient, unpredictable and varying climatic conditions (Index Mundi, 2015). These environments are characterized by a lack of adequate water in spring and summer periods when evaporation and transpiration are rising rapidly when crops are in the later stages of development, which results in a terminal drought. There is also a problem of frost when the air temperature drops to 2°C or less. Damage to crops from frost may occur at any stage of development but is particularly damaging around the flowering window. These constraints result in a serious dilemma for growers who must decide whether to delay anthesis to avoid frost damage or flower as early as possible in order to escape the effects of terminal drought (Richards, 1991). Thus, it is important that barley cultivars demonstrate an adaptation with appropriate rates of development across the heterogeneous environments; development rates that occur sufficiently late to avoid frosts but sufficiently early to avoid terminal heat stress in a given environment.

2.2 The relationship between barley phenology and abiotic stresses, quality and yield

Phenology characterises the developmental life cycle events of plants and how these events are influenced by seasonal and inter-annual variation in climate as well as habitat factors

(Juskiw et al., 2001; Wilczek et al., 2010). In barley, the time of developmental stages such as spikelet initiation and duration of grain development can seriously influence yield and quality. These stages are regulated by environmental factors such as temperature or growing degree-days (*GDD*), duration and intensity of light, nutrition and husbandry techniques (Landes et al., 1989). Heading date is important in adapting barley genotypes to different stresses such as heat stress, waterlogging, salinity and drought. Heat stress can quickly deplete the available moisture through high rates of evapotranspiration and ultimately lead to terminal drought (Hossain et al., 2012). Both heat and drought at late sowing may interfere with barley developmental processes usually from double ridge (DR) to maturity. The resultant effects of these stresses are reduction in plant height, dry matter accumulation and grain yield (Hossain et al., 2012). On the other hand, low temperature at early growth stages (Zadoks GS10) may be required for vernalisation especially for winter barleys to flower. Apart from the optimum conditions, poor biomass accumulation and significant yield losses are attributed to extreme conditions, high or low temperatures, drought or waterlogged anaerobiosis and other soil-related problems (Cattivelli et al., 2010). Advances in crop phenology and modelling have helped with the understanding of how to assess biomass partitioning and effects of abiotic stresses in crops (Juskiw et al., 2001). Modelling has also helped understand the effects of different environments and sowing dates on growth and development of barley crops.

Many scoring systems for plant growth stages have been developed to describe phenology in cereals (Landes et al., 1989). The most widely used are Feekes scale (Landes et al., 1989) and Zadoks scale (Zadoks et al., 1974). Most scales describe only morphological traits (Juskiw et al., 2001) and very few of them describe the apical developmental processes especially in barley (Banerjee et al., 1965). The vital developmental stages that have significant effects on

yield and quality include DR (Zadoks GS30), the construction phase, which includes stem elongation (GS31), heading and anthesis (GS51 and 61 as in barley) and grain filling stage (Bezant et al., 1996; Laurie et al., 1995). DR and terminal spikelet (TS) can only be detected through destructive means (Fig 2.1). There have been no consistent reports on the use of correlated traits for the determination of the flower initiation stage (GS30), though in some cases surrogate traits such as number of leaves on the main stem have been used to determine this stage (Boyd et al., 2003).

The DR stage is a prerequisite step for predicting flowering date and crop yields and has been an important trait for improving crop productivity and adaptation (Yin et al., 2005a). A reduced construction phase (GS31 to 65) and grain filling period often reduces yield (Schelling et al., 2003). The increase in the rate of grain filling has a positive correlation with grain weight (Al-Karaki, 2012). In the High Rainfall Zones of Australia (along the coast in regions with over 550 mm annual rainfall), wheat crops with more GDD between GS31 and GS65 (i.e. stem elongation to anthesis) were shown to have greater grain yield (Acuna et al., 2015).

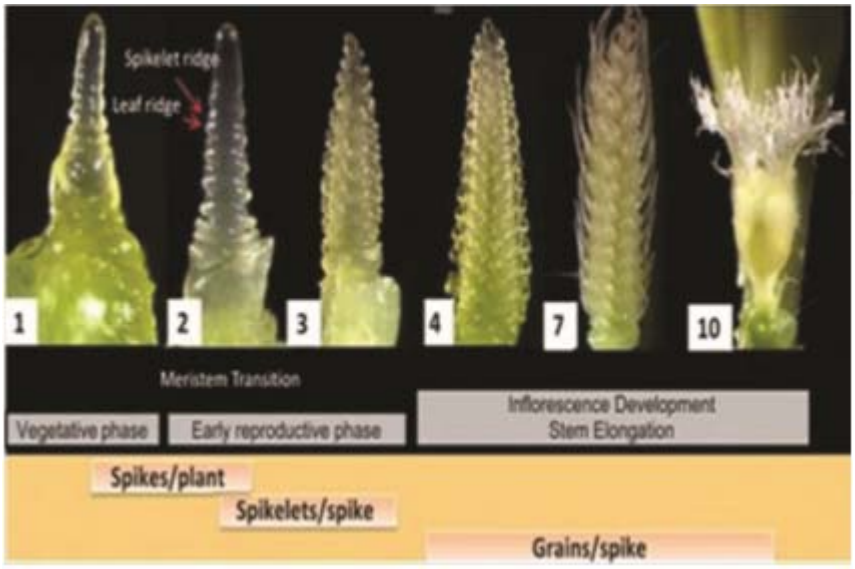

Figure 2.1 Development stages from double ridge to awns emergence (Klepper et al., 1983).

2.3 Factors affecting phenological development in barley

Barley development has three main stages: germination/emergence to double ridge/stem elongation (GS10 to GS30/31), stem elongation to heading/anthesis (GS30/31 to GS51/61) and heading to physiological maturity (GS51 to GS 94) (González et al., 2002; Hossain et al., 2012; Snape et al., 2001). The first state, the basic vegetative phase, is the stage for the production of phyllochrons, roots and tillers. The agronomic significance of this stage is the generation of enough biomass for livestock feed especially for dual purpose genotypes (Redmon et al., 1996). The second stage involves the termination of vegetative growth GS29. This stage signifies reproductive growth, spikelet initiation and the onset of stem elongation (Baker et al., 1983) and is required for the production of a higher number of spikelets which directly link to grain yield (González et al., 2003). The third stage is grain filling, influencing grain size and weight (Whitechurch et al., 2002). This stage is essential for fostering greater yield and increasing

grain quality. All the three stages are regulated mainly by physio-genetic, environment as well as management.

Important environmental factors affecting barley developmental stages include temperatures and photoperiod (Hossain et al., 2012; McMaster et al., 2003; Miralles et al., 2001), both varying simultaneously in field conditions. This variability can considerably affect developmental events that determine flowering time and consequently yield (Miralles et al., 2001). Temperature is very important to plant physiological processes (Went, 1953), especially for the variation in days to spikelet initiation (Hay et al., 1998), days to heading and days to flowering (anthesis) in cereals (Roberts et al., 1988; Yin et al., 1997). Hay et al. (1991) and Ellis et al. (1988) also reported that the rate of primordia initiation in barley has a linear relationship with average daily temperature. The rate of initiation of organs in barley also increases linearly with temperature; the optimum temperature for organ development is 25-30°C (Hay et al., 1998). However, low temperature is required in some cereals to stimulate flowering (vernalisation); this term has been used as the basis for classification of barley into winter and spring types. Variation in phyllochrons (period between successive leaf appearance on the main stem) in different genotypes of barley is more likely due to the variation in the combinations of temperature and photoperiod (Juskiw et al., 2001). Growing-degree-days (GDD) are often used for measuring pheno-stages in barley. GDD is the accumulation of the mean daily temperature above a base temperature (0°C in the case of barley) (Bauer et al., 1992; McMaster et al., 2003). Below 0°C, the development of the crop will cease while above 0°C the growth will increase linearly with temperature (McMaster et al., 2003; McMaster et al., 1997; Went, 1953). Using zero as the base temperature (Tb = 0°C) in wheat, Acuna et al., (2015) identified an ideotype which may develop sufficient tiller

numbers at 650°Cd to harvest about 400–500 heads/m^2. The same ideotype had a construction phase duration (CPD) within 800–1200°Cd that allowed heading to occur after risk of frost had declined and allowed partitioning of more assimilate to developing grain. Miralles et al. (2001) reported the GDD that ranged between 950-2000°Cd and 1300–2100°Cd from sowing to flowering in both barley and wheat, respectively (Miralles et al., 2001). This result reflects the high variability in GDD for flowering time in barley, indicating that yield might be manipulated using this characteristic.

Photoperiod is also a key environmental factor that affects the development of barley especially in temperate countries. Day length has a predictable pattern that drives evolutionary plant responses. (Kikuchi et al., 2009). Photoperiod can significantly influence the duration of vegetative, spikelet initiation and stem elongation periods in wheat and barley (Miralles et al., 2000). The long day length of higher northern and lower southern latitudes causes both photoperiod-sensitive and photoperiod-insensitive cultivars to flower early. The winter type responds strongly to long days, while maturation of spring types varies depending on the selection criterion (Turner et al., 2005). For example, in Western Europe and parts of North America, short days increase the duration of vegetative growth of spring-sown barley. This lengthens the time for biomass accumulation and hence increases yield (Turner et al., 2005) under temperate growing conditions. Australia has a unique environment that differs from other barley growing regions in higher latitudes (Boyd et al., 2003), where day length is much shorter in winter, but considerably longer in summer (Table 2.1). However, there is variation in the extent of sensitivity of Australian genotypes to photoperiod. The baseline for the sensitivity ranges from 8 to 10 h of exposure, below which no flowering initiation occurs,

while the upper limit ranges from 13 to 18 h (Roberts et al., 1988). The sensitivity of both vernalisation and photoperiod starts immediately after plant emerges (Roberts et al., 1988).

Management is also an important factor that affects phenology in barley; important factors include sowing date, fertiliser application, irrigation and other management practices. Matching the phenology with an appropriate sowing window allows growers to better manage climate risks that are particularly pronounced in Australia, where early sowing may expose the heading of spring barley to frost, while late flowering and terminal drought can curtail grain filling and hence reduce yield (Richards, 1991).

Table 2.1 Sunshine duration across Australian regions and their coordinates

Region	**Coordinates:**	**Seasonal sunshine hour duration**			
		Winter: Jun-Aug	**Spring: Sept-Nov**	**Summer:- Dec-Feb**	**Autumn: Mar-May**
NT, Katherine	14.465°S, 132.26° E	11-12	12-13	12-13	11-12
Perth	31.95°S, 115.87°E	10-12	11-14	12-14	10-13
Carnarvon	24.88°S, 113.66°E	10-12	11-14	12-14	10-12
Victoria Mallee	35.11°S, 142.36°E	9-11	11-14	12-14	10-12
Ravensthorpe WA	33.32°S, 119.82°E	9-11	11-14	12-14	10-12
SA Wimmera	37.82°S, 140.78°E	9-11	11-15	13-14	10-12
Adelaide	34.93°S, 138.60°E	9-11	11-14	13-15	10-13
Brisbane NSW	27.47°S,153.02° E	10-12	11-15	12-14	10-13
NSW Queensland	20.92°S, 142.70° E	9-12	11-14	12-14	12-14
Launceston, Tas	41.44°S, 147.14°E	9-11	11-15	13-15	9-13

Plant growth and development are affected by high temperatures and water stress in late sowing of spring barley (Hossain et al., 2012; Ram et al., 2010), high temperatures at early sowing in Punjab of India (Ram et al., 2010) and low temperatures with early sowing in Russia (Hossain et al., 2012). In the same experiment with early sown crops, low tillering (as a result of low temperatures) caused a significant reduction in grain yield (Hossain et al., 2012). Similar results were reported by Ram et al., (2012), in that very early sowing of spring barley affected tillering capacity. Sowing date was also found to have significant effects on the phenological

development of cereals crops, especially during the GS31 (stem elongation phase) in wheat (Kiss et al., 2014).

2.4 Physiological and molecular mechanisms for heading date and their effects on grain yield and quality

Heading date (spikelet emergence, GS51) is a complex trait in barley that has direct impact on grain yield and quality. The mechanisms regulating this trait are so complicated that there has been no final conclusion on the specific number of genes that are involved and their interactions (Hay et al., 1998). Although the expression of this trait is governed by complex factors such as genetics, physiology and environment (Boyd et al., 2003) neither the plant breeder nor the physiologist can clearly explain their interactions. For example, physiologists have not answered some unresolved developmental issues such as the regulation of the developmental rate within an environment and the cause of the transition between one-growth stages to another. Equally, crop breeders need to account for the gene functions, the number of the genes and their interactions that are involved in expression of heading date (Hay and Ellis, 1998).

2.4.1 Genetic regulation of heading date

Barley improvement dates back to its domestication period. However, significant yield improvements began only in the 1950s and greater advances have been made through the application of more advanced plant gene technologies (Thomas, 2003). Studies have been conducted on the genetic improvement, which include the determination of genotypic and phenotypic variability in days to flowering and growth phases, as well as comparing different sets of cultivars in barley (Borràs et al., 2009; González et al., 2002) and wheat (Whitechurch et al., 2007). Recent advances in the area of molecular breeding using highly polymorphic

molecular makers such as simple sequence repeat (*SSR*) and single nucleotide polymorphisms (*SNPs*) have also led to significant progress in the improvement of yield and quality in barley. These markers are being used to tag genes or quantitative trait loci (QTLs) that are of economic importance, offering promises in their use in marker-assisted selection (MAS) (Jahoor et al., 2005). Among all the markers, *SNPs* and *SSR*s are believed to be best suited for the use in marker assisted breeding (Gupta et al., 2000). They have been used to assess most of the genes in barley and other cereals through complementary DNAs (*cDNAs*), expressed sequence tags (*ESTs*) and sequenced PCR amplicons that provide use of SNPs in protein encoding transcribed genes.

Research into the effects of environment on heading date was initiated following the reports of Garner et al. (1920). They showed that variation in flowering date among different genotypes of barley were due to the effects of seasonal variation, location and sowing date; these findings were also confirmed by Boyd et al. (2003). To characterise such variation, genotypic differential response to photoperiod, vernalisation and other environmental conditions have been conducted. Of the three groups of genes that are responsible for variations in heading date, two groups have major on reproductive development (Table 2.2). These include *Ppd* (photoperiod) (Kikuchi et al., 2009; Laurie et al., 1995; Ren et al., 2012; J. Wang et al., 2014) *Vrn* (vernalisation) (Distelfeld et al., 2009; Karsai et al., 2005) and *earliness per se* (*Eps*) (Bullrich et al., 2002; Lewis et al., 2008). *Eps* determines the time and duration of the reproductive phase. Of the three genes, only Eps gene acts independently of vernalisation and photoperiod (Bezant et al., 1996; Laurie et al., 1995; LÜ et al., 2013). All the *Vrn* genes have been cloned (Faure et al., 2012; Turner et al., 2005; Yasuda et al., 1993).

Table 2.2 Flowering genes with major effects, encoded protein, putative functions, habit and responses and references

Gene	Encoded protein	Function	Habit and sensitivity	Reference
Ppd-H1	Psuedo-response regulator (PRR)	Photoperiod sensitivity and flowering time	Ppd-H1 (Photo-sensitive) Ppd-H1(photo-insensitive)	Turner et al 2005, Alqudah et al 2014, Hill et al 2016
Ppd-H2	HvFT3	Promote flowering under SD	Ppd-H2 (Photo-response at SD) ppd-H2 (delay flower in SD)	Cockram et al (2015), Alqudah et al 2014
Vrn-H1	MAD-BOX (BM5A)	Flowering promoter in response to vernalisation and spike development	Vrn-H1 (Spring) vrn-H1 (winter)	Cockram et al 2007, Hill et al 2016, Sasani et al (2009)
Vrn-H2	ZCCT-Ha/Hb	Flowering repressor, growth rate	Vrn-H2 (winter) vrn-H2 (spring)	Cockram et al 2007b, Sasani et al (2009)
Vrn-H3	HvFT1	Flowering promoter and integrator in response to vernalisation	Vrn-H3 (spring) vrn-H3 (winter)	Karsai et al 2008, Faure et al 2007

All the three groups of genes have been well researched in wheat, although there is no conclusive evidence that *Eps* genes have been cloned in all cereals. These genes begin regulation of development from emergence at GS10 (Bezant et al., 1996). Hence, there is a lack of information on the genetics, physiological, biochemical functions and location of the *Eps* gene in all cereals but especially in barley (Boudiar et al., 2016; Cockram et al., 2007; Faure et al., 2012). Unfortunately, access to the barley genome has not been straight forward because the genome consists of a large number of repetitive sequences (Appels et al., 2003; LÜ et al., 2013). Scientists have explored opportunities in colinearity of the genes among the cereals (LÜ et al., 2013; Cockram et al., 2007; Faricelli et al., 2010; Valárik et al., 2006) for marker design and elucidation of the effects of the genes on yield and quality and their interaction with the environment in barley (LÜ et al., 2013). It is, however, important to understand the behaviour and interaction of these *Eps* genes with different environment and management practices. Particularly in Australia, variable climates make production decisions and genetic improvement for crop adaptation difficult (Meinke et al., 1993). Evidence of deferential genotypic response to ambient temperature and other climatic parameters is limited in barley (Boyd et al., 2003) thus; the knowledge of genotype x environment or QTL × environment as well as management (G×E×M) interactions is required to help obtain higher grain yields and quality (Hammer et al., 2014). The use of crop simulation modelling to predict expression of complex crop traits under diverse environments has provided plant breeders and farm managers with opportunities to make crucial decisions such as matching the choice of genotype to an appropriate sowing window, in different environmental conditions (Chapman et al., 1993). Therefore, integration of experimentally determined genetic responses to photoperiod, vernalisation and earliness per se will complement plant breeders in their use of genetics and molecular tools in the prediction of flowering time and the

understanding of how these genes affect grain yield and quality in different climates and with different management.

2.4.1.1 Photoperiod genes (Ppd)

The photoperiod pathway is generally classified into two components: the circadian clock and the photoperiod clock regulators (Imaizumi et al., 2006). The clock is the receptor of light stimuli perceived by phytochromes (*phyA* to *phyE*) for red and far red and cryptochromes (*cry1* and *cry2*) for blue light receptors (Imaizumi et al., 2006). Temperate cereals including barley, are quantitative long-day crops (Boyd et al., 2003); however, some varieties differ in their response to photoperiod (Dofing, 1995), as mentioned above. Photoperiod sensitivity can significantly influence the duration of both vegetative and stem elongation periods in wheat and barley (Miralles et al., 2000). There is evidence that specific stages in floral development in wheat and barley may also be sensitive to light intensity, with heavy shading during the later stages of ear development may result in the infertility of the spike (Aspinall et al., 1963). As a result, flowering in photo-sensitive plants like barley may be entirely inhibited if the light intensity is reduced sufficiently during long periods because of the low level of availability of carbohydrates within shaded plants (Aspinall et al., 1963). Therefore, genotypes vary in the photoperiodic threshold below, which flowering initiation will not take place.

In barley, two photoperiod genes influencing flowering time are *Ppd-H1* on chromosome 2(2HS), which regulates flowering time under long days (Wang et al., 2014; Ren et al., 2012; Wang et al., 2010; Laurie et al., 1994; Börner et al., 2002) and *Ppd-H2* on chromosome 5(1HL) that regulates flowering time under short days (Bezant et al., 1996; Börner et al., 2002). *Ppd-H1* is a *pseudo-response* regulator gene (*HvPRR37*) (Alqudah et al., 2014) and is the major

controller of heading date when crops are exposed to long days (LDs). Therefore, the spring varieties of barley consist of this dominant allele. However, when present the recessive allele *ppd-H1* is the major cause of the reduction in photoperiod response in spring types and is hence the reason for late flowering in LDs (Turner et al., 2005). Reduced photoperiod responsiveness of the *ppd-H1* mutant is explained by altered circadian expression of the photoperiod pathway gene *CONSTAN*S and reduced expression of its downstream target, *HvFT1*, which is controlled by *HvCO1*, a key regulator of flowering (Faure et al., 2012; Laurie, 1997). *EARLY FLOWERING3 (ELF3)*, which is also a member of the circadian clock genes, regulates flowering under the influence of photoperiod (Boden et al., 2014). This gene also has a loss-of-function mutant in plants (e.g. barley and some legumes) that causes early flowering in short days (SDs) as well as in LDs. In the same way as the *ppd-H1* operates, the recessive mutant *eam8* (*mat-a*) has a loss of function characteristic (Faure et al., 2012) that leads to the insensitivity to photoperiod and thus can cause early flowering in both SDs and LDs (Faure et al., 2012; Boden et al., 2014). However, *eam8* is significantly involved in the expression of *HvFT1* (a flower initiator) which is also an allelic variant at *Ppd-H1* locus (Faure et al., 2012). Similarly, the barley *elf3* mutant helps in the expression of the *GA20oxidase2 gene*, which causes the production of gibberellin (GA) in the apical meristems under SDs. Production of GA activates the early-flowering *elf3* in SDs in the absence of the *FT1* gene (Boden et al., 2014). The second photoperiod gene (*Ppd-H2*) responds to SDs. *Ppd-H2* acts similarly to *HvFT3* when exposed to SDs. In an experiment conducted using Morex and Steptoe populations, the expression of the *HvFT3* was not found in the Steptoe genotype (which has the *ppd-H2*) but was found in Morex (which has the *Ppd-H2* gene). Therefore, *HvFT3* has been named as the candidate for *Ppd-H2* (Casao et al., 2011). In spring barley, the

Ppd-H2 allele is the major actor regulating flowering but is rarely found in commercial winter types (Casao et al., 2011).

Many other QTLs have been identified from different populations. Ren et al., (2012) detected a major QTL under 18-h photoperiod in glasshouse experiments and mapped the QTL to the Xp12m50B199–Xp13m47B399 interval of flanking markers on chromosome 4H, which accounted for 77% and 38% of phenotypic variation for long photoperiod response in Australia and China, respectively. The gene, *eam7*, showed a stronger effect on flowering time with 55-day and 18-day shorter compared to *Ppd-H1* (chromosome 2H) and *Ppd-H2* (chromosome 1H) (Stracke et al., 1998). Another *eam7* gene determining photoperiod insensitivity under SDs was identified on the short arm of chromosome 6H near the centromere (Stracke et al., 1998). This gene was 6.7 and 13.0 cM away from two flanking markers Xmwg2264 and Xmwg916, respectively. Environmental factors also had a significant effect on the expression of two different QTLs, for flowering time, which were mapped to chromosomes 1HL and 7HS when the population was grown under long photoperiod conditions. However, no QTL were detected in the same population when they were grown under short photoperiod conditions (Börner et al., 2002). The QTL for heading date are often linked with yield in barley (Bezant et al., 1996; Karsai et al., 1999). These could be the part of the reason why most of these genes have a highly significant effect on several agronomic traits, such as biomass accumulation including grain yield and grain quality in barley (Laurie et al., 1995; Karsai et al., 1999). The photoperiod responsive genes in wheat were found to be in homologous series to genes on barley chromosome 1H, 2H (Snape et al., 2001).

2.4.1.2 Vernalisation genes (Vrn)

Vernalisation is the requirement for prolonged low temperature to advance flowering in cereals and depends on the growth habit (such as spring or winter types). The winter types of barley require cold exposure before flower initiation, typically below 10°C for a period between 4 and 6 weeks (MASWHEAT, 2015), depending on base temperature as defined above. In contrast, the spring types have minimal low temperature dependency and are usually insensitive to vernalisation and Short-day photoperiod (von Zitzewitz et al 2005). This behaviour is characteristic of many temperate cereals like barley (von Zitzewitz et al 2005; Sasani et al., 2009) and associated with the capacity of a genotype to survive the cold winter during vegetative stages (von Zitzewitz et al 2005; Hayes et al., 1997; Francia et al., 2004). Barley is an excellent model for genetic analysis of low temperature tolerance in fall-sown cereals (Francia et al., 2004). Its responses to vernalisation have been observed to vary greatly among genotypes and between growth phases (Miralles and Richards, 2000; Francia et al., 2004).Vernalisation in cultivated barley is mainly controlled by three major *Vrn* genes (Snape et al., 2001), *Vrn1, Vrn2* and *Vrn3* (Distelfeld et al., 2009), or *HvVrn1*, *HvVrn2*, *HvVrn3* (Sasani, et al., 2009), or *Vrn-H1*, *Vrn-H2* and *Vrn-H3* (Karsai et al., 2005). The *Vrn-H1* (also named as *Sgh2* or *Sh2*) is located in the middle of the long arms of 5H (Cockram et al., 2007; Sasani, et al., 2009). The *Vrn-H2* (*Sgh* or *Sh*) is found on chromosome 4H (Cockram et al., 2007), while the *Vrn-H3* (*Sgh3* or *Sh3*) is found on 1H (Karsai et al., 2005). *Vrn-H1* translates the fruit-like MADS-box transcription factor, which is an ortholog *APETA-LA1* gene (Yan et al., 2003). The allelic difference at this gene locus is essential for flowering in temperate cereals (Yan et al., 2003; Sasani, et al., 2009; Trevaskis et al., 2003) and therefore, it is one of the major determinants of vernalisation requirement in barley and wheat (Loukoianov et al., 2005). Within the locus, the allele that is responsible for the spring growth habit is *Vrn-H1* (the

dominant one) (Fu et al., 2005), while the recessive allele accounts for genetic regulation of the winter habit (Fu et al., 2005; Shitsukawa et al., 2007). A large deletion in the first intron of *Vrn-H1* locus in the dominant allele is responsible for the null response to vernalisation in spring barley and wheat (Fu et al., 2005; Shitsukawa et al., 2007), while no deletion within intron 1 was observed in the winter habit types possessing recessive *vrn-H1* allelic loci (Cockram et al., 2007; Sasani, et al., 2009; Fu et al., 2005).

The second locus is the *Vrn-H2* (*Sgh2* or *Sh2*) which encodes for the zinc finger-*CCT* (*ZCCT-H*) transcription factor (Fu et al., 2005) and is also vernalisation dependent. A partial or total deletion of part of this locus has been shown to cause a non-functional mutation of the gene and a recessive form is responsible for the spring growth habit in both barley and wheat (Yan et al., 2003; Loukoianov et al., 2005; Cuesta-Marcos et al., 2010). However, it is necessary to understand that the effects of *Vrn-H2* under field conditions can only be verified using a variety of sowing dates (Karsai et al., 2005). The authors further stated that the gene does not affect heading date when crops were autumn sown.

The third locus is the *Vrn-H3* (*Sgh3* or *Sh3*) on chromosome 1H (Laurie et al., 1995; Snape et al., 2001; Sasani, et al., 2009) and later on 7HS (LÜ et al., 2013; Yan et al., 2006). This gene is an ortholog of the FT gene in Arabidopsis and *HvFT1* gene (Wang et al., 2010), which responds to vernalisation in both barley and wheat. A study conducted by Yan et al., (2006) showed that homologous spring barley with dominant *Vrn-H3* allele had an increase in *HvFT* transcript rapidly, while the recessive genotype *vrn-H3* had low *HvFT* transcript without vernalisation. A strong relationship was found between the *Vrn-H3* and Ppd genes as the *HvFT* was observed to be very low in SD and upregulated in LDs (Yan et al., 2006). Finally, for a given winter genotype to respond to vernalisation, it must have all three genes (*vrnH1:Vrn-H2:vrnH3*). All

other combinations are reported to be spring types (Karsai et al., 2005; Sasani et al., 2009; Cuesta-Marcos et al., 2010). These three loci (*VRN-H1, VRN-H2* and *VRN-H3*) interact in an epistatic fashion to determine vernalisation sensitivity (Cuesta-Marcos et al., 2010). This interaction depends on daylength (SD or LD) (Hill et al., 2016). As mentioned above, the response of the barley genotypes is determined by the circadian clock regulators including *Ppd-H1* and *Ppd-H2* genes. Usually, in winter genotypes under LD, the dominant *Ppd-H1* allele is upregulated (Alqudah et al., 2014) and the activation of the expression of the non- mutant recessive *vrn_H3* which occurred after vernalisation (Hill et al., 2016; Turner et al., 2005). *Vrn-H2* delays flowering before vernalisation under LD by inhibiting or deregulating the expression of *Vrn-H3* which is the central integrator of photoperiod and vernalisation pathways (Pidal et al., 2016) (Fig, 2). However, after vernalisation or in SD *Vrn*-H1 represses *Vrn*-H2 allowing for the upregulation and expression *Vrn-H3*, thus opens the pathway and rapid progression of floral initiation (Distelfeld et al., 2009; Pidal et al 2016). There is no information of the alternative pathway through which *Vrn-H2* regulates *Vrn-H1* other than through the repression of *Vrn-H3* (Pidal et al., 2016). *Ppd-H2* can also influence flowering by upregulating *Vrn-H3* in LD when *Vrn-H2* is depressed or deregulated (Casao et al., 2011) as showed in (Fig 2.2) and can directly activate flowering under SD (Faure et al., 2007).

Since there is a form of homogeneous genetic system for all the cereals with a high degree of synteny (physical co-localisation of genetic loci on the same chromosome in an individual or species), the results of one species are frequently applicable to other members of the cereal family (Dubcovsky et al., 1998; Mahfoozi et al., 2000; von Zitzewitz et al., 2005), including barley. Consequently, the cloning of the candidate genes in diploid wheat (*Triticum monococcum*) of *VRN-A*^{m1} and *VRN-A*^{m2} (Yan et al., 2003; Trevaskis et al., 2003; Yan et al.,

2004) and hexaploid wheat (*T. aestivum*) of *VRN-1* has considerably increased our understanding of the genetics of vernalisation in barley (von Zitzewitz et al 2005).

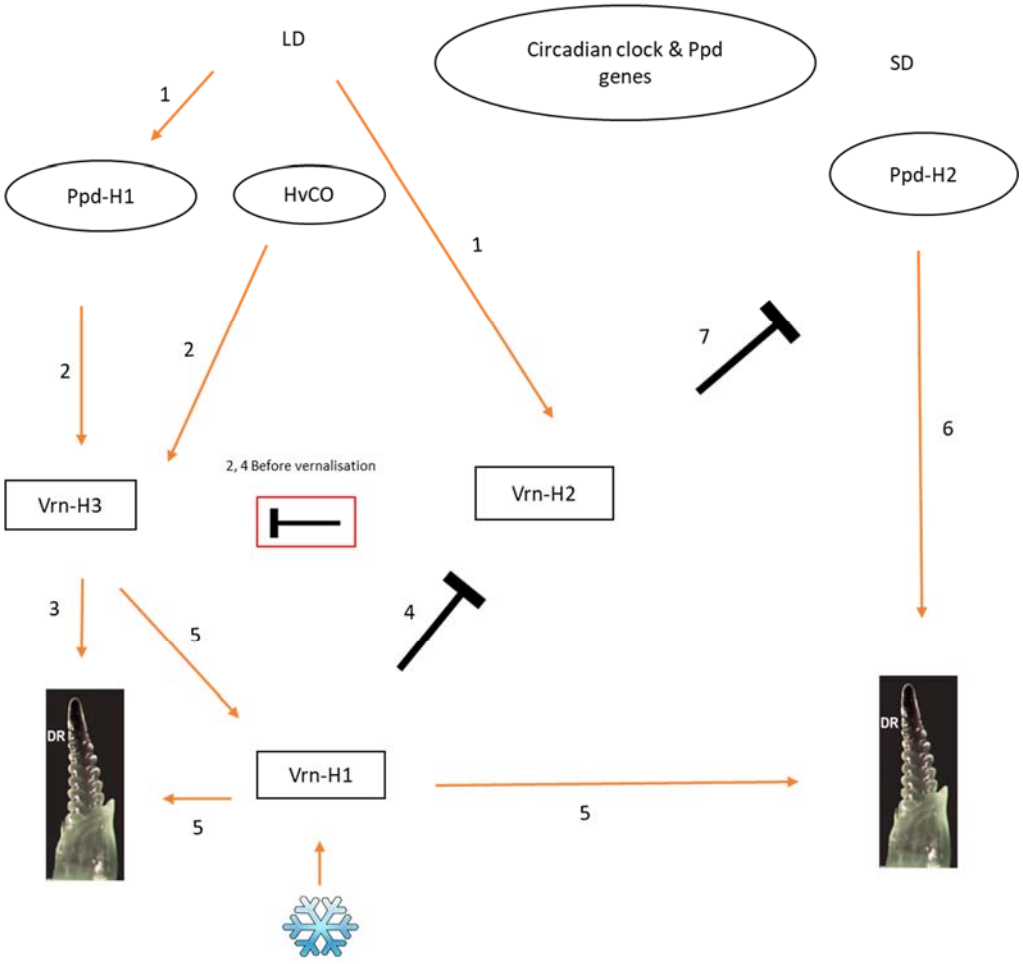

Figure 2.2 Genetic path ways and interactions of major genes regulating the timing and duration of individual reproductive phases in barley. Circadian clock regulators in ovals. Vernalisation genes in boxes. The regulated and depressed pathways are shown by red arrows and T-ends respectively under long day (LD) and short day (SD). The numbers on the pathways represent the literature which supports the experimental evidence. [1]Hemming et al (2008), [2]Hill et al (2016), [3]Mulki et al (2016), [4]Pidal et al (2016), [5]Yan et al (2006), [6]Faure et al (2007) and [7]Casao et al (2011)

2.4.1.3 Basic vegetative phase BVP (Earliness per se, Eps) genes

Early growth plasticity of barley is determined during the vegetative phase (Slafer, 2003), which has extensive genetic diversity. One of these genes is the *Earliness per se, Eps*. This gene regulates the basic vegetative phase (*BVP*) in barley and influences the time and duration of growth stages from DR (*Zadoks* GS30) to grain filling stage (*Zadoks* GS70) (Bullrich et al., 2002; Lewis et al., 2008). Expression of this gene can only be fully observed when all the other sources of the variations in flowering time have been fixed, i.e. when the environmental stimuli such as exposure to adequate vernalisation and photoperiod requirements have been met by the plants (Faricelli et al., 2010; Valárik et al., 2006; Appendino et al., 2003; Zikhali et al., 2014). In addition, *Eps* is also actively involved in the fine-tuning of the flowering time in cereals including barley (Valárik et al., 2006; Griffiths et al., 2009).

Various authors have identified the *Eps* gene in all the chromosomes of common wheat (Valárik et al., 2006) and barley (Laurie et al., 1995; LÜ et al., 2013; Cockram et al., 2007). Recent advances in molecular genetics have shown that the location and physiological effects of the *Eps* gene on yield and quality in barley are limited (LÜ et al., 2013). Since most of the cereals share similar genetic synteny (LÜ et al., 2013), it could be assumed that results from studies on reports on wheat could be applied to barley. Efforts to identify the markers linked to the genes and their locations are underway. In wheat, an RFLP marker (wg241) was observed to be linked to *Eps-A^{m1}* gene on 1H (Valárik et al., 2006). The gene was found to be 0.7 cM distal to wg241 and 1.4 cM proximal to the barc287 markers (Bullrich et al., 2002; Valárik et al., 2006). Among the three markers reported in *Brachypodium* and wheat plants, two were identified to be *molybdenum transporter 1* (*MOT1*) (transcriptional regulator) and

filamentation temperature-sensitive H4 (*FtsH4*), respectively (Faricelli et al., 2010; Faricelli et al., 2008). These makers were linked to the *Eps* gene and were proposed as candidates for *Eps-A^{m1}* on chromosome 1H (Faricelli et al., 2010; Faricelli et al., 2008). The predicted *MOT1* protein showed differences in the amino acid between the parent lines in which the effects could not be predicted (Faricelli et al., 2008). Thus, any future steps to clone the *Eps-A^{m1}* gene should include the generation of *mot1* and *ftsh4* mutants and the completion of the *T. monococcum* physical map to test for the presence of additional candidate genes.

2.4.1.3.1 Effects of Eps gene on developmental phases

Most previous research conducted has centred on the effects of Eps gene on the flowering time (Lewis et al., 2008; Yan et al., 2003; Cockram et al., 2007) with fewer focussing on the variation in the duration of each of developmental stages (Lewis et al. 2008; Slafer et al., 1996; Hoogendoorn, 1985). For example, a study conducted by Lewis et al. (2008) using single seed decent and near isogenic lines (NILs) observed a significant interaction between the *Eps* gene and the timing and duration from vegetative to flowering phase, especially from double ridge to terminal spikelet stage in diploid wheat. The interaction showed that the NIL genotypes with the early allele, *Eps-e*, had the transition to DR stage 35 days earlier (67% less) than the genotypes with the *Eps-1* alleles. The *SSD* genotypes had highly significant differences ($P < 0.0001$) in both heading time and number of spikelets per spike between *Eps* alleles (*eps-e* and *eps-1*). The genotypes with the late allele (*Eps-1*) flowered 61 days later than those with *eps-e* alleles and produced a mean of 8.7 more spikelets for each spike which was a 56% increase across temperatures (Lewis et al., 2008). However, results of Valárik et al., (2006) and Zikhali et al., (2014) showed only a few days of differences (from 1 to 5 days) in flowering time between a pair of NILs and their recombinant inbred lines (RL) in both wheat and rice. In

addition, no significant interaction (P=0.67) was observed between *Eps* genes and the stem elongation stage (Lewis et al., 2008), which is the beginning of construction phase. In addition, temperature had no significant effects on the gene determining spikelets number per spike (Lewis et al., 2008). Contrary to these findings, Slafer et al., (2005) observed that lengthening the duration of the stem elongation phase, without modifying total time to anthesis, could increase the number of grains/m^2 and consequently the number of grains per unit land area. In general, variation in both flowering time and spikelet number per spike could be due to pleiotropic effects of a single gene or to the effect of tightly linked multiple genes with additive effects (Valárik et al., 2006).

2.4.1.3.2 Effects of temperature on expression of the Eps genes

Temperature is the main environmental factor affecting *Eps* genes in barley and wheat (Lewis et al., 2008; Zikhali et al., 2014). Differences exist among genotypes carrying *Eps-e* for early heading and *Eps-1* alleles for late heading in wheat (Bullrich et al., 2002). The *Eps* genes show significant interactions with temperature (Lewis et al., 2008; Bullrich et al., 2002). The genotypes with *Eps-1* alleles had no interaction with temperatures (21°Cd difference), while lines carrying the *Eps-e* allele had a shorter thermal time to heading at 16°C than at 23°C (336°Cd difference) (Lewis et al., 2008). The same study further showed that the thermal time to flowering for the genotypes with the *Eps-e* gene was approximately 1557^{o}Cd. This GDD is approximately 1118^{o}Cd less than the thermal time for the late genotypes (2675^{o}Cd) with the *Eps-1* gene. Slafer et al. (1996) used four spring wheat varieties and six differential temperatures (10-25^{o}C) to study the effect of temperature on growth stages. They showed that the developmental phases of individual genotypes were most sensitive to temperature from sowing to anthesis. This variation can be attributed to the allelic diversity at *Eps* locus in

the lines studied. A comparable detailed study of genetic variability of the earliness per se genes (*Eps*) is required for a more precise analysis of their effects on developmental stages and temperature sensitivity in barley.

2.4.1.3.3 Effect of sowing date on expression of the Eps genes

To maximise yield potential in any environment, cultivars must have an appropriate flowering window and life cycle duration in the target environment (Snape et al., 2001; Bullrich et al., 2002). Sowing date is an important factor that governs flowering period, the timing of which needs to escape biotic and abiotic stress. Of the three major genes, *Ppd*, *Vrn* and *Eps*, only *Eps* genes do not respond to the differential to vernalisation or photoperiod and still control timing and duration of flowering independent of these stimuli (Snape et al., 2001).

2.4.1.3.4 Effect of the Eps gene on grain quality

Yield and quality are important complex traits in any breeding programme. Improvement of these traits is very difficult to achieve due to their genetic, physiological and physical complexity. Grain quality could either be physical, such as size, hardness and lustre, or nutritional such as malting quality. The relationship of *Eps* gene on heading, spikelets number and number of grains per spike has been shown to be correlated with yield (Lewis et al., 2008). However, grain quality can also be improved by manipulating the *Eps* gene loci (Zikhali et al., 2015) to improve the physical traits such grain weight or hardness. Experiments conducted by Herndl et al. (2008b) indicated that with a shorter pre-anthesis period, the relationship between yield and protein is always negative. Crop breeders often focus on increasing yield with little attention on quality traits; thus, there is less information on the effects of the *Eps* gene on quality traits. Heading date is a polygenic trait, controlled by *Ppd* (Castro et al., 2008; Takahashi and Yasuda, 1971), vernalisation (Hay and Ellis, 1998; Roberts et al., 1988; Ellis et

al., 1988; Castro et al., 2008) and *Eps* genes (Castro et al., 2008; Gallagher et al., 1991). These genes interact in an additive nature (cumulative effects of non-allelic genes to a quantitative trait) (Castro et al., 2008). The genetic analysis of *Eps* showed that it can be simply inherited as a Mendelian inheritance, but molecular analysis has not been able to identify an appropriate molecular marker to determine its location (Boyd et al., 2008).

2.4.2 Modelling the effects of phenological genes on crop yield

Crop modelling in agriculture has been used as a physiological framework to undertake simulation of dynamic crop phenology that supports crop improvement programmes (APSIM, 2015). Physiologically-sound simulation tools will provide quantitative assessments of crop development and yield relative to the genotype, climate, soil and management in sustainable farming systems (Keating et al., 2003). These tools can provide ex-ante impact assessments of research outcomes across a wide range of environments (Slafer, 2003). This is particularly necessary for Australia where a highly variable climate poses challenges for production (Meinke et al., 1993) and may undermine genetic breeding efforts. In southern Australia, temperature has increased by 0.9^{o}C since 1910 per annum and severe heat and drought spells are occurring more frequently. Total annual rainfall and frost events may also increase in some temperate regions such as Tasmania (CCIA/CSIRO, 2015). Other reports indicate a 2–5% reduction in rainfall across most parts of the country (Anwar et al., 2015). Harrison et al. (2016) emphasise the need for serious attention on the impacts of climate stress on plant phenology. Globally, climate change will further increase temperatures, modify the amount and distribution of rainfall and consequently reduce the probability of reliable food and forage production, thereby causing a significant threat to food security and improved livelihood (Harrison et al., 2014). More than 30% barley yield loss will be attributed to climate change

as a result of drought and heat stress by 2050 (Van Gool and Vernon, 2005). Despite this, there has been little research work to determine how the climate change will broadly affect whole farm systems (Harrison et al., 2016; Phelan et al., 2015), particularly systems farming barley.

Mathematical functions have long been used as tools to simulate crop phenology to predict the effects of climate events on yield and quality (Bouman et al. 1996; Yin et al., 2005). These tools help explain the interaction of some of the complex traits related to development and growth and their interaction with the environment. For example, ecophysiological quantitative equations were used by Yin et al., (2005) to describe the response of flowering to photoperiod and temperature to predict days to heading and yield in diverse conditions. The equations were used as empirical and mechanistic models to provide an important framework for simulating a number of events in crop growth, especially predicting heading date (Yin et al., 2005; Bogard et al., 2014). The empirical models are often based on accumulation of GDD adjusted by vernalisation and photoperiod (Bogard et al., 2014; Asseng et al., 1998), while mechanistic models are based on the production of leaves and floral primordia at the apexes (Jamieson et al., 1998).

Four important phenology models: 3s-beta-model, 3-plane-linear-model, modified-rice-clock model (m-RCM) and a logistic model were developed and evaluated in rice (Yin et al., 1997; Yin et al., 2005). All models were able to predict the flowering time in different environments although with varying degree of precision. Model parameter values from reciprocal transfer experiments also resulted in realistic differences in flowering time across all the genotypes in different environments. The models were able to partition variation due to environment and that of the genotypes. Thus, ecophysiological model can be important in dissecting the

relationship between genotype and phenotype (Yin et al., 2005). Chapman et al., (1993) developed a mechanistic model called *QSUN* to estimate growth, development and yield of a diverse range of genotypes of sunflower under varied environments. Their model was able to account for leaf area index (r^2 = 0.65), total biomass (r^2 = 0.96) and grain yield (r^2=0.93) when tested against actual phenological data. *QSUN* was also used to analyse the production risk of sunflower grown in highly variable subtropical environments to undertake decisions such as the choice of an adapted cultivar and appropriate sowing window to obtain higher yields (Meinke et al., 1993). Another dynamic model was used to investigate the causes and impact of climate change on peanut production in Northern Australia (Meinke et al., 1995). The model was used in conjunction with the information of district yield to study long-term production risk. The study indicated that the stabilisation of the above-average yield, which was due to stable summer rainfall events, was responsible for the rapid expansion of peanut industries at that time. Such studies assist in gaining better understanding of complex GxExM and the identification of traits required to manage crops in variable and changing environments (Meinke et al., 1995). Thus, choice of an appropriate simulation model for predicting phenology is essential for studies that purport to examine cultivar suitability to production environments and how phenology is affected by planting time or other tactical management (Meinke et al., 1993; Bogard et al., 2014).

2.4.2.1 The agricultural production systems simulator (APSIM)

The Agricultural Production Systems Simulator (APSIM) is a cropping systems simulation model that combines several decision-support tools. APSIM may be used for prediction of how traits like heading date impact grain yield and biomass of different crop genotypes under alternative environment and management conditions and also to consider the long-term

consequences of cropping systems on soil conditions (Keating et al., 2003; Holzworth et al., 2014). APSIM may also be used to increase the understanding of factors influencing heading date of barley when grown under field conditions (Juskiw et al., 2001). APSIM can also be used to describe genetic parameters regulating phenology with the function of daily temperature and photoperiod to predict flowering time and consequently their effects on yield and quality (Yin et al., 2005). The major climatic challenges facing barley production are water stress coupled with heat stress during spring and summer and frost events in winter and early spring. District yield records showed about 85% yield loss due to frost events in Australia (Zheng et al., 2015). A later study with more information was conducted to gauge the impact of frost on grain production in Australia (Zheng et al., 2015b) wherein APSIM was used to simulate the effects of frost on wheat production areas across Australia. The model predicted increased frequency of frost events in the Australian wheat belt (the main barley production regions in Australia) but also an increase in the mean temperatures with significant yield loss. Zheng et al. (2015b) concluded that breeding for frost tolerance could give about 20% yield advantage. It is likely that some of this frost tolerance could be enacted by manipulation of flowering time. The barley module of APSIM (*APSIM-Barley*) simulates phenology on a daily time step. The module uses inputs of weather including solar radiation, maximum and minimum daily temperature, rainfall and vapour pressure (APSIM, 2015b). The module has 11 growth stages, from sowing to harvest (GS0-GS100) (APSIM, 2015b). Manschadi et al., (2006) used APSIM to assess barley growing patterns under different environment and management. In their study the model was able to account 91% and 82% of the variation for biomass accumulation at maturity and grain yield. The majority of crop models are constrained in predicting both the physical and cryptic (nutritional) grain quality such as grain size and grain-N content (Nuttall et al., 2016), although the model has been used to account

for both above and below ground biomass, growth, water, N uptake and leaching (Asseng et al., 1998). The same model was used to explain some quality parameters such as grain size and grain protein concentration (Asseng et al., 2008) and improvements in the model demonstrate its reliability in simulating grain protein content under a wide range of conditions (Asseng et al., 2002). Several years ago, a barley production simulation model called *QBAR* was developed to help identify appropriate management options such as sowing date to increase yield in environments with prevailing heat and drought stress (Goyne et al 1996). *QBAR* can simulate phenology, soil water, leaf area, biomass accumulation and yield of barley (Goyne et al 1996). *QBAR* was later modified to *APSIM-Barley* (Goyne et al 1996), and in validation experiments, the model accounted for 91% and 82% of the variation of biomass accumulation and grain yield, respectively (Asseng et al., 2015). *QBAR* has also been used to account for the effects of extreme climatic events, frost and terminal drought on yield and yield components, of which paddock-based crop models could not explain (Barlow et al., 2013). The authors proposed that *QBAR* can be used to determine the best management decisions such as sowing date to obtain highest grain yield even in the events of frost and water stress.

2.4.2.2 Genotype by environment by management interactions (G×E×M)

Information on the target environment for which crop cultivars are to be improved is vital to plant breeders (Shorter et al., 1991). This is because a higher genetic (G) diversity for flowering time has been reported in diverse environments (E) of barley growing regions where frost and drought events limit crop growth as well as inappropriate agronomic management (M) to improve crop growth and yield (Hammer et al., 2014). This concept can be extended to include both abiotic factors such as soil, water stress and waterlogging as well

as induced stresses due to abiotic (salinity) and biotic factors (pests, weeds and diseases). Environmental factors can be classified into two types: (1) micro-environmental factors such as year-to-year variation in rainfall, drought conditions, pest incidence and (2) macro-environmental factors, which include soil type and management practices (Wu and O'Malley, 1998; Bondari, 2003). Association between environmental and genotypic effects in producing a specific phenotype is termed a GxE interaction (Bondari, 2003). Hence, the GxE interaction determines the adaptability and suitability of a specific genotype to a range of environments. Environment could also be a time boundary, such as a year for annual crops (FAO, 2009).

Hence, matching heading date to diverse environment may give a large GxE interaction (FAO, 2009). Variation in developmental stages (particularly from DR to the grain filling stages in barley) is influenced by GxE (Garcia et al., 2002). For highly variable arable cropping regions like those found in Australia, there is a need for specific adaptation (genotype response and better performance in a specific environment) arising from GxE interaction (Ceccarelli et al., 1994). Löffler et al. (2005) used a crop model index approach to account for the GxE interaction effect in US maize breeding trials. A linear generic model was used to analyse the interaction of 96 genotypes to different environment (Yin et al., 2005). The model was able to explain 81% of the total variation in heading date across the environments. The introduction of molecular markers has aided our understanding of the effect of individual gene or QTL effects rather than the cultivar (Yin et al., 2005). Yin et al. (2005) used a four-parameter ecophysiological model to predict grain yield when QTL-based data inputs were used. The model when used together with the QTL map was able to sufficiently predict days to flowering in barley (Yin et al., 2005), suggesting that the model could be used to help breeders in Australia to adapt new varieties in different environments.

Recent advances in plant breeding combined with dynamic models are now allowing partitioning of the effect of management in the G×E approaches (Hammer et al., 2014). Higher genetic gain in yield has been attributed to better understanding of G×M interaction effects in maize crops, where the progressive yield increase in the US has been associated with superior genotypes being grown at higher density (Duvick, 2005). Another example is the choice of a combination of non-tillering genotypes (G) and row spacing (M) in drought prone land can help realise sustainable production and additional value in obtaining moderate yield instead of complete crop failure due to limited availability of water (Hammer et al., 2014; Hammer, 2006). Another study was conducted to examine the performance chickpea genotypes under two different managements, irrigation and rain-fed management systems. The study showed highly significant yield differences among genotypes and between the two management practices for all the important traits. The study also revealed that both yield and yield components were improved by an average of 48% increase in the number of pods per plant, 36% in total dry weight and 17% in grain yield in the management involving irrigation (Bakhsh et al., 2007).

Hence, the use of models capable of accounting for GxExM interactions in agricultural systems can be a powerful tool to better understand environment-specific, complex gene expressions. *APSIM-Barley* has been used to describe broad adaptation of barley genotypes in anticipation frost or water stress across Australia. In another experiment, leaf area and yield of Baudin, Flagship, Buloke and Capstan, barley cultivars were assessed (Hunt and Poole, 2010). The model also reasonably explained the relationship between the leaf area duration and yield as influenced by weather (Hunt and Poole, 2010). Matching specific genotypic traits to

management option within a target (specific) environment will assist breeders in trait selection and the design of their breeding program.

Chapter 3 A regulator of early flowering in barley (*Hordeum vulgare* L.)

Abstract

Heading date (HD) of cereals is an important trait for adaptation to diverse environments and is critical for determining yield and quality and the number of genes and gene combinations that confer earliness in barley under short days is limited. In this study, a QTL for early flowering was identified from the cross between an Australian malting barley cultivar and a Chinese landrace. Four sets of near isogenic lines (NILs) were developed with a QTL located on chromosome 5H at the interval of 122.0–129.0 cM. Further experiments were conducted to investigate how this gene was regulated by photoperiod using the NILs with three sowing dates from autumn to summer. The NILs carrying the earliness allele were significantly earlier than the late genotype at all sowing dates. This gene was different from previously reported vernalisation genes that are located at a similar position as no vernalisation was required for all the NILs. The difference between this gene and *Eam5* (*HvPHYC*) locus which also located between two co-segregated markers (3398516S5, 122.5 cM, and 4014046D5, 126.1 cM), is that with the existence of *Ppd-H1* (*Eam1*), Eam5 has no effect on ear emergence under long days while the gene from TX9425 still reduced the time to ear emergency. The locus showed no pleiotropic effects on grain pasting properties and agronomic traits except for spike length and number of spikelets per spike, and thus can be effectively used in breeding programs. The array of early heading dates caused by interactions of *Eam5* gene with other maturity genes provides an opportunity to better fine tune heading dates with production environments, which can be critical factor in barley breeding.

3.1 Introduction

Barley is an important cereal crop grown worldwide under a wide range of environments (Cockram et al., 2007). The broad adaptation of this crop to varying climatic and regional conditions is in part caused by the diversity in flowering time (anthesis, Zadoks GS61) or heading date (HD, Zadoks GS51) or tipping (awn emergence, Zadoks GS 49) (Alqudah et al., 2017; Cuesta-Marcos et al., 2008). These terms are often used interchangeably by many scientist (Alqudah et al., 2017). The main factors affecting HD are photoperiod, vernalisation, temperature and management (Harrison et al., 2014; McMaster et al., 2003). These factors provide the physiological and genetic basis for variations in the duration of developmental stages, such as double ridge (DR), terminal spikelet (TS), heading, anthesis and grain filling (Boyd et al., 2008; Boyd et al., 2003; Juskiw et al., 2001). Genotypes vary in their photoperiodic response (Boyd et al., 2003; Roberts et al., 1988), with temperature being very important to plant physiological processes (Went, 1953) especially variations in duration to spikelet initiation (Hay et al., 1998), heading and flowering (anthesis) in cereals (Roberts et al., 1988; Yin et al., 1997). It follows that a linear association generally describes the relationship between cumulative temperatures over the growing season and heading/anthesis in barley (Ellis et al., 1988; Hay et al., 1998; Hay et al., 1991) and in many other cereals. To initiate flowering, winter barley requires vernalisation, i.e. exposure to prolonged temperatures below 10˚C for a period between 4 to 6 weeks (MASWHEAT, 2015; Sasani et al., 2009; von Zitzewitz et al., 2005).

Early maturity of cool-season cereals like barley under short-day environments is vital in many grain producing regions of the world. Barley grown in Eastern Asia has evolved unique earliness mechanisms that stimulate early ear emergence under short days even when

vernalisation is not necessary. These mechanisms may involve several maturity genes interacting together in either additive, epistatic or pleiotropic way in order to regulate earliness. Apart from these allelic and no-allelic interactions, intra-locus mutations at *Vrn* loci, *Eps* and H*vPHYC* loci are responsible for earliness in both barley and wheat (Mizuno et al., 2016; Pourkheirandish et al., 2007) with a rich allelic variation at *Vrn-H1* or/and *HvPhyC* (Alqudah et al., 2014). Our understanding of these interactions is gradually improving as we learn more about their mechanisms of expression and that some of these genes are functional under long days. Four major genes are reported to be responsible for vernalisation. These include *Vrn-H1* (Sgh2), *Vrn-H2* (Sgh1), (Sgh3) and Vrn4 on chromosomes 5H, 4H, 7H and 5H, respectively (Kato et al., 2003; Sasani et al., 2009; Trevaskis et al., 2003). These four loci interact in an epistatic fashion to determine vernalisation sensitivity in barley and wheat (Cuesta-Marcos et al., 2010). For example, the winter barley cultivars which are responsive to vernalisation have *vrn-H1_Vrn-H2_vrn-H3* haplotype (Cuesta-Marcos et al., 2010; Karsai et al., 2005; Takahashi et al., 1971) and more recently were found to have the recessive mutant *vrn4* (Kippes et al., 2016). A model of heading-time regulation in both photoperiod groups (*Ppd-H1*; *ppd-H1*) under long day condition was proposed by Alqudah et al (Alqudah et al., 2014).

Heading date in barley is regulated by photoperiod response genes; the first identified being *Ppd-H1* (Eam1), which is a pseudo-response regulator gene (*HvPRR37*) that is effective under long days and is located on chromosome 2H (Alqudah et al., 2014; Faure et al., 2012; Ren et al., 2012; J. Wang et al., 2014). The second photoperiod gene, *Ppd-H2 (HvFT3)*, is located on chromosome 1H and regulates flowering time under short days (Börner et al., 2002; Wang et al., 2010).

Earliness in intrinsic or per se genes determine the time and duration of reproductive phases (Bullrich et al., 2002; Lewis et al., 2008). These QTL manifest their expression after all sources of variation in basic vegetative period (*BVP*) or maturity-related traits (such as vernalisation and photoperiod) have been met (Appendino et al., 2003; Faricelli et al., 2010; Zikhali et al., 2014). Thus, *Eps* QTL are important in fine-tuning HD and anthesis in barley (Griffiths et al., 2009; Lewis et al., 2008; Slafer, 2003). *Eps* QTL have significant effects on the time and duration of reproductive phase and spikelet number (Lewis et al., 2008) which directly affect the grain yield (Griffiths et al., 2009). *Eps* QTL also have significant effects on grain protein (Herndl et al., 2008), which is inversely related to the starch (Fox et al., 2009; M. Gupta et al., 2010). The *EARLY FLOWERING3* (*ELF3*) locus regulates flowering under the influence of photoperiod (Boden et al., 2014). The recessive allele (*elf3, eam8, mat-a*)of this gene in barley causes early flowering in both short days (SDs) and long days (LDs) (Boden et al., 2014) and an insensitivity of barley to photoperiod (Boden et al., 2014; Faure et al., 2012).

Apart from the *Eps* QTL, an early maturity factor in chromosome 5H was reported by Wexelsen (1934). The gene belongs to the family of photoreceptors, phytochromes, which helps plants to perceive, interpret, and translate light signals that modulate and synchronize their growth and development in any given environments (Biyashev et al., 1997; Mathews, 2010). These phytochromes are involved in plant metabolism including flowering, shade avoidance, dormancy, and germination (Franklin et al., 2009). Close linkage to the rough awn trait demonstrates that *Eam5* is likely the locus reported by Laurie et al. (Laurie et al., 1995) and later identified at the *HvPHYC* locus after which Szucs et al. (Szucs et al., 2006) mapped to a similar position as that of *Vrn-H1*. *HvPHYTOCHROMEC (HvPHYC*) is reported to be a candidate gene to *Eam5* (Pankin et al., 2014). Nishida et al. (2013) found that a mutation at

HvPHYC, due amino-acid substitution in the GAF domain, influenced HD under LDs. It was later found to affect ear emergence under long and non-inductive short days (Hill et al., 2016; Pankin et al., 2014). Thus, *Eam5* likely interacts with many photoperiod response loci under an array of photoperiods. In addition, a *casein kinase alpha* (*HvCK2α-5H*) gene is also reported to be closely linked to the *Vrn-H1* (Nishida et al., 2013). This gene encodes the α subunit of *CK2* protein and regulates flowering, which is found in cereals including rice, wheat and barley under both short and long days (Nishida et al., 2013).

Beside the photoperiod and vernalisation genes, which have been cloned, there is little information on the genetics, physiological and biochemical functions of other genes regulating early ear emergence in barley. For instance, *HvPHYC* gene has been recently fine mapped but there is discrepancy surrounding the expression and interaction of this gene with the environment. Further work is required to determine their quantitative effects (Comadran et al., 2012). This manuscript provides some details about interactions of one component of the circadian clock with various photoperiod regimes in essentially fixed genetic background in barley.

From thousands of F10 recombinant inbred lines from the cross of TX9425 and Franklin, we have identified another early flowering allele that may be distinct from other QTL on chromosomes 2H, 3H and 6H which were reported earlier (Wang et al., 2010). Four pairs of near isogenic lines (NILs) were developed to investigate: 1) the location of this early flowering gene; and 2) the effect of this gene on agronomic traits, yield components, and grain quality.

3.2 Materials and methods

3.2.1 Development of near-isogenic lines

NILs were selected from over 1,000 F9 derived F10 recombinant inbred lines (RILs) of the cross of TX9425 and Franklin. Franklin (Shannon/Triumph) is a late maturing malting variety from Australia (DPIT, 1989) with the seeds being sourced from the University of Tasmania and the Australian Grains Genebank. TX9425 (Taixing 9425) is a Chinese landrace, which has semidwarf and early maturity genes, most likely the dominant alleles of the Ppd-H1, the spring *Vrn-H1* allele and the recessive allele of the *Eps5HL* (Wang et al., 2010). From the RIL population four lines segregating on heading date were further selfed to produce homozygous early and late lines, leading to four pairs of the NILs. They are *Eps5HL-116 (-E/-L*), *Eps5HL-317-1(-E/-L)*, *Eps5HL-317-2 (-E/-L)*, *Eps5HL-322 (-E/-L)* with–E carrying earliness allele. All NILs have both the spring *Vrn-H1* allele as vernalisation is not needed to all lines and *Ppd-H1* since the segment containing this gene is from TX9425 (Fig 3.1)

3.2.2 Genotyping of the NILs

Genomic DNA of NILs was extracted from the leaf tissue of four-week old seedlings, according to the plant DNA extraction protocol for DArT analysis (https://www.diversityarrays.com/files/DArT_DNA_isolation.pdf). The two parental cultivars and four pairs of NILs were genotyped with DArTseq (http://www.diversityarrays.com/dart-application-dartseq). Around 10,000 polymorphism molecular markers with known positions were chosen for comparing the differences between NILs and the relationships to their parents.

3.2.3 Field experimentation

Field experiments were conducted at Mt Pleasant Laboratories, at the Tasmanian Institute of Agriculture (TIA) in Launceston, Tasmania (Latitude: -41.4702 Longitude: 147.1392), where

the day length ranges from 9 to 15 hours (Fig 3.2). The four pairs of NILs and both parents were sown in tanks (1.50 x 3.0 x 1.0 m) with a spacing of 20.0 cm x 7.0 cm. Tanks were filled with sandy loam soils and fitted with irrigation facilities to avoid any water stress. The NILs/parents were grown in a randomized complete block design with three replications.

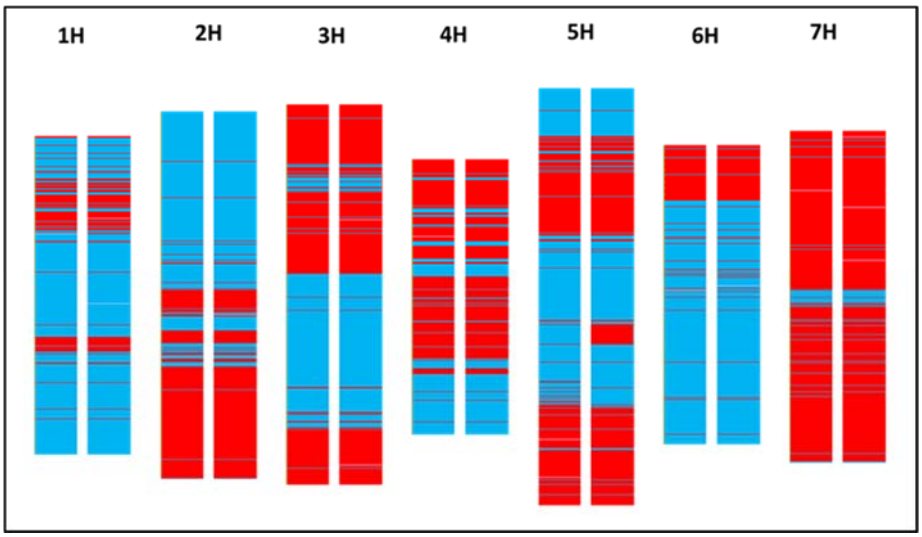

Figure 3.1 Comparison between genotypes of near isogenic line (Eps5HL-317-1-E, left, vs Eps5HL-317-1-L, right) from the cross of TX9425/Franklin. Red: Franklin genotype; blue: TX9425 genotype. The major difference is on 5H at the position of 122 to 129 cM (circled).

Three different sowing dates, 15/January/2015 (SD1, summer), 13/March/2015 (SD2, summer/autumn) and 15/May/2015 (SD3, autumn), were selected to represent the photoperiods/ temperatures (Fig 3.2).

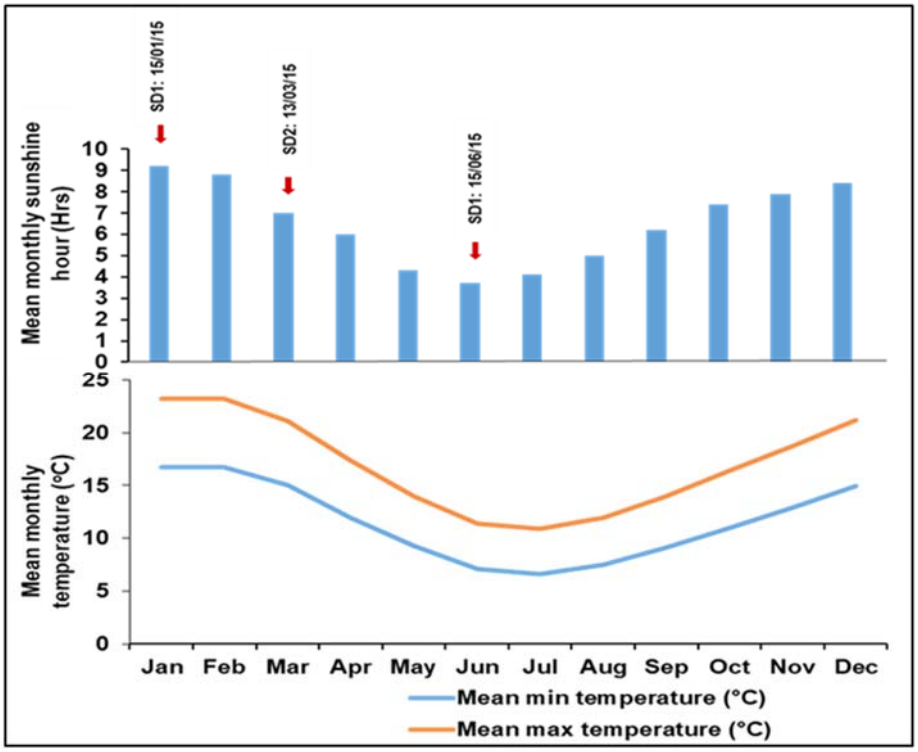

Figure 3.2 Mean monthly temperatures and day length hours per month in 2015 for Tasmania. Arrows are three different sowing dates.

Agronomic practices such as fertilizer rate and regular weeding were similar to local practices. Traits measured shown in Table 3.1 include: heading date (HD) (when the first spikelet emerged in one of the tillers of 50% of the plants) in calendar time, growing degree days to heading date (GDD), plant height (PH), number of nodes on the main plant, peduncle length, spike length, awn length and number of fertile spikelets per spike. Harvested grain was tested for pasting properties. Climatic data were taken from the nearest meteorological station, and GDD accumulation was calculated following the classical method:

$$GDD = \sum_{i=1}^{n} \left[\left(\frac{T_{min} + T_{max}}{2} - T_b \right) \right]$$

Where *n*= number of days taken for a particular growth stage to be accumulated, and T_{min}, T_{max} = minimum and maximum daily air temperatures in °C, respectively, and T_b = base temperature threshold for barley, which is 0 °C (Nuttonson, 1956)

Table 3.1 Traits scored and their abbreviations

Traits	Symbol	Unit	Description
Heading date	HD	d	Number of days from sowing to when fifty percent of the spike appears
Thermal time (growing degree days)	TT/GDD	^{o}Cd	Accumulated thermal time from day 0 of the sowing day to current growth stage
Plant height	PH	cm	Measured from collar to the peak of the awns
Spike length	SpkL	cm	Measured from the base of the spike to the tip
Spikelet number	SpkN	Spk/spike	Number of spikelets in a spike
Peduncle length	PedL	cm	Measured from the last node to the base of the spike
Rapid Visco-analyser Unit	RVU	RVU	An RVU is approximately equal to 10 cP.
Peak Viscosity	PV	RVU	Highest viscosity during cooking
Time to Peak Viscosity	TTPV	min	Time taken to reach the peak
Trough	TR	RVU	Lowest viscosity after cooling started
Breakdown	BD	RVU	Peak viscosity minus trough (PV-TR)
Final Viscosity	FV	RVU	Maximum viscosity after the temperature had returned to 50 oC
Setback	SB	RVU	Final viscosity minus trough (FV-TR)
Pasting Temperature	PT	^{o}C	Temperature when the rate of increase in viscosity reaches 11.5 RVU in 0.2 min

3.2.4 Analysis of pasting properties

The determination of pasting properties was conducted according to the methods described by (Zhou et al., 2005; Zhou et al., 1998). After harvesting and threshing, the samples in each NIL pair were air- dried. 10.0 g of grains were sampled from each of the NIL pairs in each replication and ground in a Cylotech 1903 Mill. 4.0 g of the flour was slurried into 25.0 g of 0.1M of silver nitrate (AgNO3) solution in an aluminium canister.

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0.1M of silver nitrate (AgNO3) solution in an aluminium canister. The slurry was then thoroughly mixed by moving the paddle both vertically and stirring in the canister before placing it into a Rapid Visco-Analyser (RVA-4D, Newport Scientific, Australia). The RVA instrument was used to determine the pasting properties. The RVA instrument was used for 10 s at 960 rpm then reduced to 160 rpm for the remainder of the test run. The initial temperature was 50 ^{o}C, held for 1.0 minute, then heated to 95 ^{o}C for 3.7 minutes and was maintained at 95 ^{o}C for 2.5 minutes before cooling to 50 ^{o}C over 3.8 min, and finally maintained at 50 ^{o}C for 2.0 min. The measured parameters for pasting properties include: peak viscosity (PV), highest viscosity during heating; time to peak viscosity (TTPV); trough (T), lowest viscosity after cooling started; breakdown (BD), PV minus T; final viscosity (FV), highest viscosity after the temperature had returned to 50 ^{o}C; setback (SB), FV minus T; pasting temperature (PT), temperature at which the trace left the baseline (Zhou et al., 1998).

3.2.5 Statistical analysis

SAS version 9.4 was used to conduct ANOVA to estimate the significances of the differences between each of the pairs, whilst the mean of each trait within genotypes was ranked used Tukey's test (Stell et al., 1980).

3.3 Results

3.3.1 Mapping early flowering QTL

The genotyping of the four pairs of NILs was conducted using DArTseq with over 30,000 markers. Fig. 3.3 shows that for the *Eps5HL-317-1* pair, except for the 122–129 cM region of chromosome 5H, genetic background was identical for the early (*Eps5HL-317-1-E*) and late (*Eps5HL-317-1-L*) lines. Similar regions were located in the other three pairs of NILs except that the region with different background was much greater (122–140 cM) (Fig 3.3) for the

Eps5HL-322 pair. The earliness allele was from TX9425. More than 100 SNP/DArT markers co-segregated with the trait.

As reported previously (Wang et al., 2010) two major QTL for early maturity were found in a DH population originating from the cross of TX9425 and Franklin. One QTL is located on chromosome 3H that is closely linked to *sdw1* and *uzu1* genes. This early maturity allele is absent in all these NILs. The other early maturity QTL is located on chromosome 2H (most likely *Eam1*) which exits in all the NILs (Table 3.2).

Table 3.2 Summary of the allelic state of the parents (TX9425 and Franklin) and the near isogenic lines (Eps-317-1-E and Eps-317-1-L) on Chromosomes 2H and 5H. No allelic differences were detected for other major genes, including PPD-H2 (1H), VRN-H2 (4H), VRN-H3 (7H), and EPS2/EAM6 (2H).

Genotype	2H	5H
TX9425	Ppd-H1[1,2]	Vrn-H1[1], Eps[2]
Franklin	ppd-H1[1,2]	Vrn-H1[1], eps[2]
Eps-317-1-E	Ppd-H1[1]	Vrn-H1[1,2], Eps[1,2]
Eps-317-1-L	Ppd-H1[2]	Vrn-H1[1], eps[2]

[1] Wang et al. (2010); [2]Ibrahim et al. (2018)

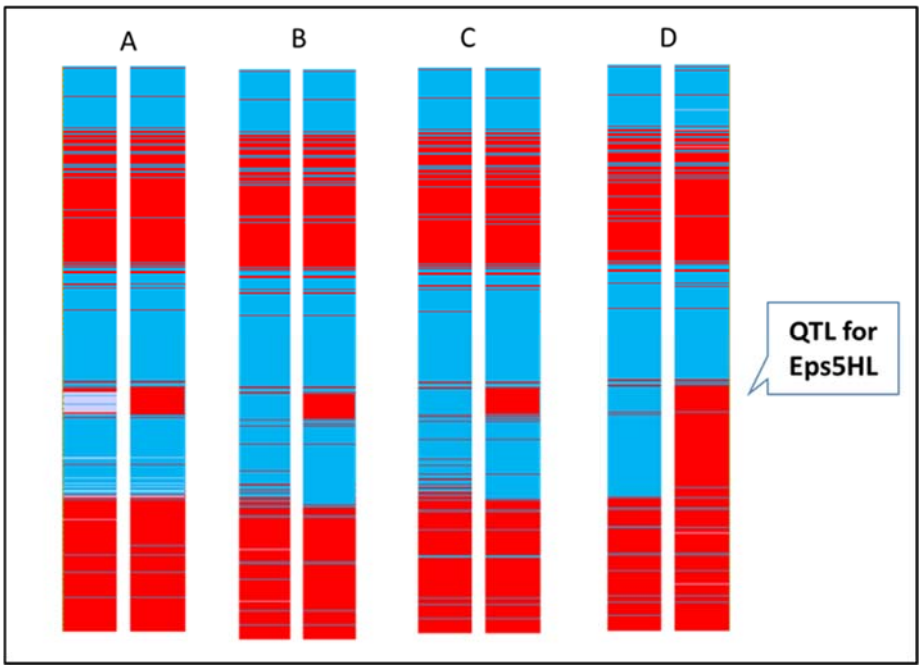

Figure 3.3 Comparison of genotypes of 5H for different pairs of near isogenic lines from the cross of TX9425/Franklin. A) Eps5HL-316-E/L; B) Eps5HL-317-1-E/ L; C) Eps5HL-317-2-E/L; and D) Eps5HL-322-E/L. Red: Franklin genotype; blue: TX9425 genotype; white: heterozygous.

3.3.2 Effects of sowing time on heading dates (HD) and GDD

Sowing dates resulted in significant differences in HD and GDD (Table 3.4, Fig 3.4). All four NILs and their parents (TX9425 and Franklin) had the fewest days to heading in SD1, followed by SD2 and SD3 (Table 3.3, Fig 3.5). Consistent differences between lines with early and late alleles existed in all the sowing dates. Heading days (HDs) for TX9425 were 41, 105 and 125 d for SD1, SD2 and SD3, respectively. HDs for Franklin were 55, 151 and 162 for SD1, SD2 and SD3, respectively. All NILs were earlier than Franklin but later than TX9425 except SD1 with the early genotypes of the NILs and TX9425 flowering at same time. HDs of the NILs with the early allele were 41, 131 and 136 for SD1, SD2 and SD3, respectively, while those with the late allele were 45, 149 and 155 for SD1, SD2 and SD3, respectively (Table 3.3, Fig 3.5).

The NILs carrying the early allele were approximately four days earlier than those with the late alleles in SD1. The differences between the two alleles were much greater with 18 and 20 days in SD2 and SD3, respectively (Table 3.3, Fig 3.5). The relative HDs for each sowing date

were similar for all pairs across sowing dates, with less difference in SD1 (Fig 3.4, Fig 3.5). When considering the effects of accumulated temperature for the HD to be expressed, similar trend was observed in the *GDD* with consistent differences among the SDs and among the genotypes except in Franklin (Fig 3.7).

Table 3.3 Means of heading dates and other different traits of two parent varieties and four pairs of NILs under different sowing dates* n = 3 (replications). Comparisons are made between the two parents and between early and late genotypes of each pair of NIL.

Genotype	HD(DAS)	GDD(°Cd)	PH(cm)	SpkL(cm)	SpkN	PedL(cm)	InterL(cm)
			SD1				
TX9425	41.0±2b	756.3±38.2b	36.0±0.7b	4.9±0.1b	20.7±1.5b	38.3±4.6a	16.6±0.8a
Franklin	55.0±4a	980.1±25.7a	47.1±2.6a	9.3±0a	24.3±1.2a	29.0±2b	16.8±2.5a
Eps5HL-116-E	42.3±1b	774.1±37b	87.9±0.5a	7.5±0.2b	22.0±1.2b	33.3±1.8a	17.6±0.8a
Eps5HL-116-L	46.7±1.3a	846.4±32a	87.7±0.8a	9.1±0.1a	25.0±1.0a	35.3±5a	15.0±1a
Eps5HL-317-1-E	41.1±1b	756.6±37.1b	83.9±0.2b	7.8±0.1b	21.3±1.5b	28.7±0.3a	19.3±1.7a
Eps5HL-317-1-L	46.0±1.7a	840.5±26.5a	95.9±4a	9.2±0.2a	25.3±1.5a	30.1±0.8a	17.7±0.7a
Eps5HL-317-2-E	41.2±2b	756.8±38b	84.2±1b	7.6±0.1b	22.4±1.5b	37.0±2.6a	19.0±1.3a
Eps5HL-317-2-L	45.3±1.6a	824±21.4a	89.7±2.5a	8.9±0.1a	25.0±1a	33.0±2.3b	16.2±1.7a
Eps5HL-322-E	41.0±1b	756.3±27b	70.0±1.1b	7.5±0.1b	21.2±2b	29.2±2a	20.2±2a
Eps5HL-322-L	45.7±1.1a	839.6±21a	76.5±1.3a	9.0±0a	25.0±1.7a	31.7±1.2a	15.6±2.5b
			SD2				
TX9425	105.0±5b	1065.2±42b	94.5±2.1a	6.0±0.3b	28.0±1.5b	25.7±2.2a	12.1±2.3b
Franklin	152.0±2.1a	1378.9±61a	84.7±7.1b	11.8±0.2a	29.0±1.1a	24.0±1.4a	14.2±2.1a
Eps5HL-116-E	131.0±2b	1238.1±23b	89.8±4.1a	10.0±0b	24.3±1.5b	30.4±2.3a	18.2±a
Eps5HL-116-L	148.9±3a	1351.0±14a	90.0±4a	11.8±0.2a	28.7±1.5a	33.4±2.3a	20.0±2.5a
Eps5HL-317-1-E	132.2±2b	1246.9±22b	97.1±8.6a	9.8±0.2b	25.3±0.5b	25.8±5a	14.2±2.2b
Eps5HL-317-1-L	149.0±3.1a	1351.6±27a	98.2±6.5a	12.0±0.2a	28.7±1.1a	24.1±9a	20.0±2.5a
Eps5HL-317-2-E	131.3±3b	1238.7±21b	82.1±2.8b	10.0±0b	24.3±0.6b	29.3±2a	20.0±2.2a
Eps5HL-317-2-L	149.0±2a	1351.6±25a	94.2±3.1a	11.8±0.3a	28.0±1.7a	29.1±2a	15.2±2.4a
Eps5HL-322-E	131.0±2b	1238.1±17b	89.4±3.3b	10.0±0b	24.3±0.6b	28.0±1.8a	18.0±1.4a
Eps5HL-322-L	149.4±2.6a	1352.0±18a	92.3±1.6a	11.8±0.3a	28.0±2a	26.8±1.3a	16.0±0.8a
			SD3				
TX9425	125.3±2.5b	925.2±28b	105.0±2.5a	6.8±0.2b	33.7±1.6b	35.0±3a	14.0±0.6a
Franklin	162.6±2.7a	1382.2±26a	86.7±3.6b	14.0±0.2a	39.3±1.5a	23.5±2b	16.0±0.2a
Eps5HL-116-E	135.0±2.6b	1029.6±24b	110.5±0.4a	11.4±0.2b	36.7±1.5b	41.0±3a	21.0±0.8a
Eps5HL-116-L	155.0±3a	1294.5±22a	110.2±0.5a	13.8±0.3a	38.3±1.4a	46.0±2a	22.0±0.7a
Eps5HL-317-1-E	134.9±2b	1029.0±21b	104.0±0.9b	11.2±0.1b	35.0±2b	36.0±1.7a	16.0±1.5b
Eps5HL-317-1-L	155.3±3a	1294.8±21a	115.0±2a	14.4±0.3a	38.7±1.6a	39.5±1.6a	22.0±2.6a
Eps5HL-317-2-E	136.1±3b	1041.8±28b	105.0±.09b	11.0±0.2b	35.3±2.5b	36.0±1.3a	17.0±1.5a
Eps5HL-317-2-L	155.1±3a	1294.6±24a	111.7±4.5a	13.9±0.2a	37.7±1.5a	37.5±1.3a	17.5±5a
Eps5HL-322-E	135.0±2.6b	1029.6±27b	98.8±0.8b	11.00.1b	33.7±2b	35.6±3b	16.0±1.4a
Eps5HL-322-L	155.1±3a	1294.6±22a	105.0±3.6a	15.3±0.2a	38.3±1.6a	42.5a	17.5±0.3a

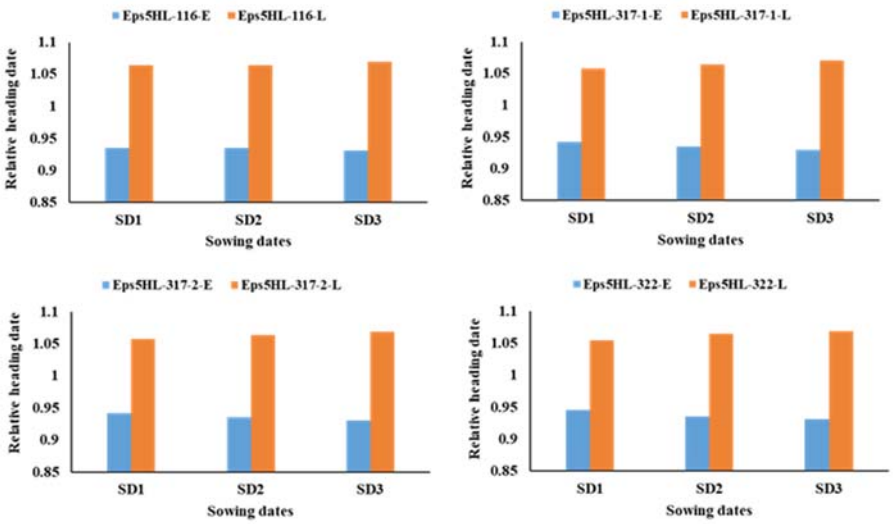

Figure 3.4 Relative heading date of different pairs of NILs, TX9425 and Franklin from different sowing dates, SD1 (15/01/2015) SD2 (13/03/2015) and SD3 (15/05/2015).

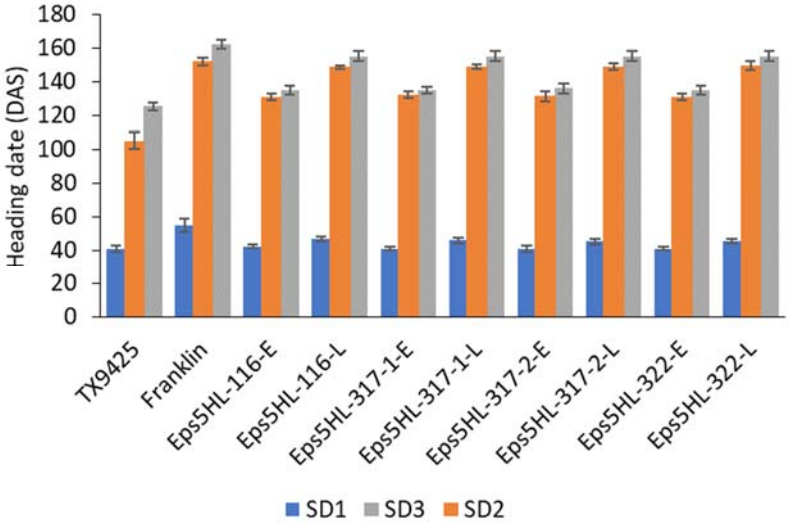

Figure 3.5 Heading date of different pairs of NILs, TX9425 and Franklin from different sowing dates, SD1 (15/01/2015) SD2 (13/03/2015) and SD3 (15/05/2015). n = 3 (replications).

3.3.3 Effects of early heading on agronomic traits and yield components

Different heading dates were associated with some of the agronomic traits and yield components. The average spike length of genotypes carrying the late allele was 1.3 cm longer than the spike length of those carrying the early allele (Table 3.3 and Fig. 3.6). Similar results

were obtained for the number of fertile spikelets per spike with SD3 having the most spikelets per spike. Genotypes carrying the late allele had more spikelets per spike that those carrying the early allele (Table 3.3 and Fig 3.6).

Figure 3.6 Morphological differences in ear emergence, maturity and spike length of four pairs of NILs carrying Eps5-317-1-E and Eps5HL-317-1-L alleles.

No significant differences between early and late lines were observed for peduncle length and internode length for most of the NILs (Tables 3.3 and 3.4). Franklin had significantly shorter peduncle length (PedL) than NILs and TX9425 in sowing dates 1 and 3, although 3172-L under sowing date 1 and 322-L under sowing date 3 also had shorter PedL. However, significant differences in plant height between early and late lines were found in three of the four pairs, with genotypes having the late allele being slightly taller than those having the early allele (Table 3.3).

Sowing dates had significant effects on overall performance of all the traits. Spike length was shorter in SD1 (7.5–9.2 cm) than that in SD2 (9.8–12.0 cm) and SD3 (11.1–14.3 cm). Similar

results were found for other traits with those from SD1 being shorter and having fewer spikelets per spike (Table 3.3 and Fig 3.6).

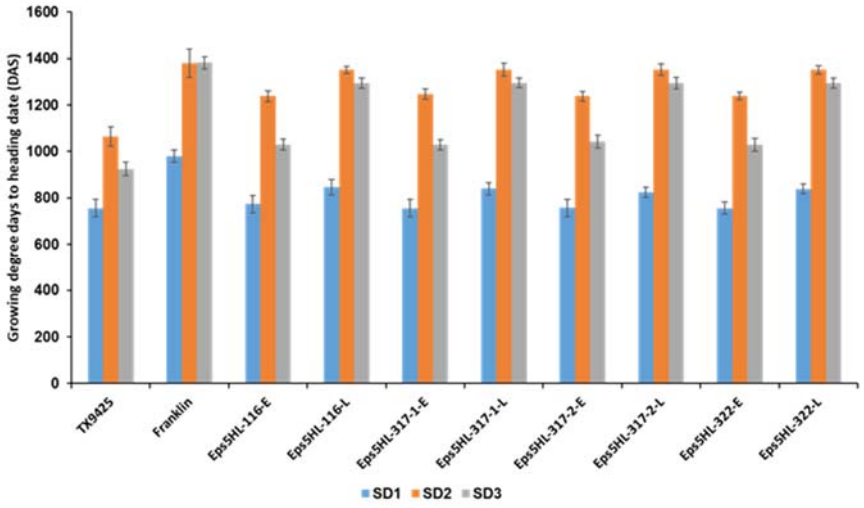

Figure 3.7 Growing degree-day (oCd) to heading date of different pairs of NILs, TX9425 and Franklin from different sowing dates, SD1 (15/01/2015) SD2 (13/03/2015) and SD3 (15/05/2015).

The differences between the early and late alleles were consistent for different SDs even though significant interactions were observed between the alleles and spike length and spikelets number per spike (Fig. 3.6).

Table 3.4 Mean square values from the analysis of variance for all the traits studied for each pair of the NILs (early and late) and the parents (TX9425 and Franklin).

S/variation	HD	GDD/TT	PH	SpkL	SpkN.	PedL	InterL
***Eps5HL-116* pair**	840.5**	90291.25**	0.067	26.94**	35.26**	1.159	0.63
SD	19522**	401622.8**	1123.43**	11.05**	335.3**	420.6**	39.6**
***Eps5HL-116* x SD**	105.5**	12505.76**	0.096	0.039**	6.12**	64.103	11.627
Err	0.0023	0.0011	5.8	0.002	0.133	9.89	1.631
***Eps5HL-317-1* pair**	840.5**	90291.25**	305.05*	27.30**	34.68**	30.86	22.819
SD	19522**	401622.8**	686.362**	11.30**	322.0**	98.562*	15.048
***Eps5HL-317-1* x SD**	105.5**	12505.76**	24.48	0.046**	3.94**	2.18	20.24
Err	0.0023	0.0011	22.74	0.004	0.18	19.6	3.75
***Eps5HL-317-2* pair**	840.5**	90291.25**	134.48*	26.94**	38.2**	19.22	0.831
SD	19522**	401622.76**	786.80**	10.98**	369.5**	55.822	0.265
***Eps5HL-317-2* x SD**	105.5**	12505.76**	10.535	0.034**	7.38**	5.01	16.433
Err	0.0023	0.0011	8.8167	0.0022	0.25	15.492	9.156
***Eps5HL-322* pair**	840.5**	90291.25**	122.201**	26.82**	34.27**	21.63	2.722
SD	19521**	401622.8**	1210.89**	11.525**	359.93**	170.897*	3.423
***Eps5HL-322* x SD**	105.5**	12505.76**	0.354	0.0705**	5.713**	9.953	21.180*
Err	0.0023	0.0011	2.706	0.0016	0.172	24.992	2.841
TXFRAN	4867.55**	495186.64**	408.98**	159.60**	72**	4.18	21.24
SD	16002**	218800.35**	4653.37**	17.85**	298.5**	18.6	3
Par*SD	438.88**	20723.39**	478.96**	2.73**	3.5	19.22	0.831
Err	8.36667	1119.057	9.311	0.033	2.4	50.822	1.265

** Highly Significant at 0.01 and * Significant at 0.05 probability level.

3.3.4 Effects of heading date on pasting properties

Fig 3.8 and Table 3.5 show that Franklin had generally higher PV, T, FV but lower BD, SB and PT values than TX9425. Both parents showed similar TTPV values. Differences were found between NIL pairs but no significant differences between early and late alleles were observed for all pasting properties (Table 3.5).

The *Eps5HL-116*, *Eps5HL-317-1* and *Eps5HL-317-2* pairs were very close to Franklin in most of the parameters while the *Eps5HL-322* pair was close to TX9425 (Table 3.6). The *Eps5HL-116* pair showed longer TTPV than both parents and other NIL pairs.

Table 3.5 Mean square values from the analysis of variance for all the pasting properties studied for each pair of the NILs (early and late) and the parents (TX9425 and Franklin).

Sources of variance	Peak Viscosity	Trough	Break-down	Final viscosity	Setback	Time to PV	P/Temp
Eps5HL- 116	1638.7	483.1	342.3	1903.9	468.9	0.002	0.053
Eps5HL-317-1	896.1	1523.1	83.1	6890.6	1934.4	0.004	2.862
Eps5HL-317-2	1031.3	1212.7	7.3	2366.1	190.9	0.014	10.580
Eps5HL-322	168.0	84.5	14.2	1098.6	1792.5	0.045	5.695
TX9425/Franklin	11630.6*	18021.9	696.9	31284.2	1817.1	0.018	28.170

* Significant at 0.05 probability level.

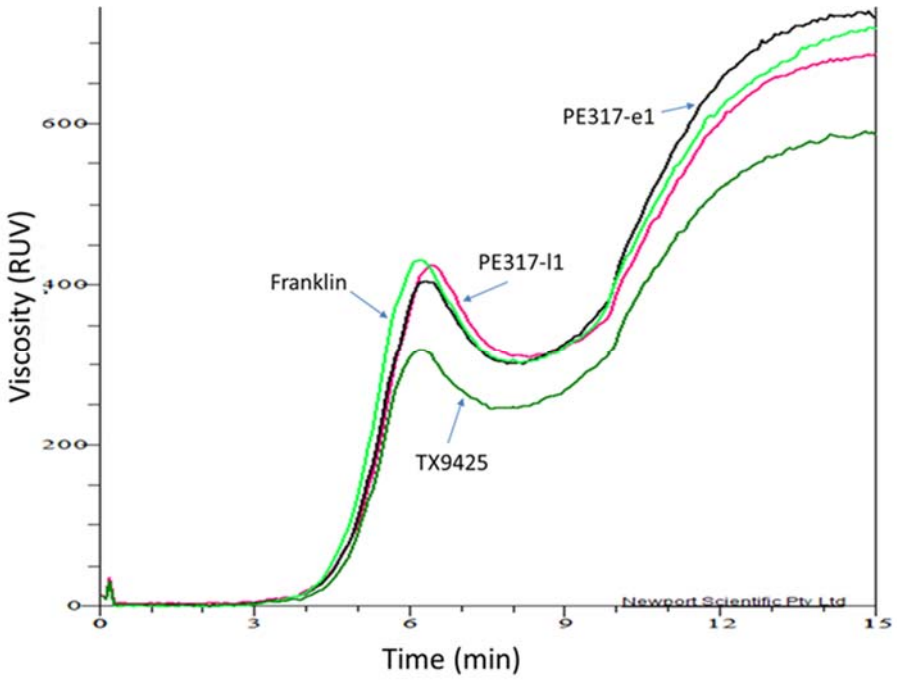

Figure 3.8 Effect of the Eps5HL locus on the starch pasting properties.

Table 3.6 Pasting properties of two parent varieties and four pairs of NILs. *

Variety/line	PV(RVU)	TR(RVU)	BD(RVU)	FV(RVU)	SB(RVU)	TTPV(min)	PT(°C)
TX9425	412b	310b	102a	699b	389a	6.38a	84.2a
Franklin	451a	357a	94b	715a	358b	6.38a	82.1a
Eps5HL-116-E	450a	350a	100a	731a	381a	6.51a	81.0a
Eps5HL-116-L	448a	354a	93a	720a	365a	6.56a	79.3a
Eps5HL-317-1-E	454a	345a	110a	748a	403a	6.28a	79.7a
Eps5HL-317-1-L	435a	320a	115a	695a	375a	6.24a	80.8a
Eps5HL-317-2-E	444a	321a	123a	727a	406a	6.18a	80.7a
Eps5HL-317-2-L	467a	346a	122a	761a	415a	6.27a	78.4a
Eps5HL-322-E	417a	309a	109a	701a	392a	6.27a	82.2a
Eps5HL-322-L	427a	315a	111a	677a	362a	6.42a	83.9a

*Values in same column followed by same letter are not significantly different (P=0.05). Comparisons are made within each pair of the NILs. Abbreviations are defined in Table 3.1.

3.4 Discussion

3.4.1 A heading date locus was identified in the long arm of chromosome 5H

TX9425, a Chinese landrace, has been reported to have different stress tolerances (Li et al., 2010; Li et al., 2008; Li et al., 2011; Li et al., 2009), semi-dwarf and early maturity genes (Wang et al., 2010). In this study, a different gene/QTL was identified for heading date with the earliness allele derived from this cultivar. This locus was mapped to a position of 122–129 cM region of chromosome 5H using four pairs of near isogenic lines. The heading dates of the lines with the earliness allele occurred about 20 d earlier than those with the lateness allele in the normal growing season (autumn sowing) in Tasmania. Similar studies conducted in temperate cereals such as barley and wheat showed that the influence of *Eps* genes on heading date is modulated by temperature (Appendino and Slafer 2003; Lewis et al 2008). Our results also showed that the genotypes carrying the earliness allele are less responsive to temperature compared with late ones confirming the effects of similar gene (Bullrich et al 2002) where the late allele was highly affected by the growing temperature compared with the early one. This QTL was not identified in the DH population originating from the cross of the same parents (TX9425 and Franklin), most likely due to the relatively smaller effects which are often masked by the effects of two other major QTL (Wang et al., 2010) thus making difficult for comparative analysis with other major genes. By comparing the position in physical maps (Mayer et al., 2012), the locus is situated at a similar position to *Vrn-H1*, a vernalisation gene (Alfonso Cuesta-Marcos et al., 2008) and two other flowering regulators, *HvPHYC* and *HvCK2α-5H* [58, 59].

Vrn-H1 is expressed when plants are exposed to prolonged cold temperatures (vernalisation) usually in winter cultivars to switch to reproductive phase (Hemming et al., 2009). Since TX9425 originated from East Asia and shows a spring growth habit, it likely has the spring *Vrn-H1* allele at this locus. Deletions in the promoter region within the first intron of the *Vrn-H1* gene is associated with increased *Vrn-H1* expression leading to the spring growth habit (Sasani et al 2009; Fu et al 2005). Pankin et al., (2014) reported that *Vrn-H1* locus is tightly linked to *HvPHYC* (*Eam5*) with no recombinants being detected between *Vrn-H1* and *HvPHYC* from a large BC1F2:3 population. Most of East Asian accessions have *HvPHYC* haplotypes 1, 3 and 4. These haplotypes existed in both winter and spring types even though the growth habit of most of these accessions were not defined (Pankin et al., 2014). Thus, the *HvPHYC* haplotype in TX9425 needs to be further investigated.

The first report of an early maturity factor in chromosome 5H was by Wexelsen (1934). Close linkage to the rough awn trait demonstrates that this likely the locus reported by Laurie et al. (1995) and Laurie et al., 1995) and later identified at the *HvPHYC* locus (*Eam5*). *Eam5* was mapped to the similar position of *Vrn-H1* (Szucs et al., 2006) and *HvPHYC* is reported as the candidate gene (Hill et al., 2016; Nishida et al., 2013; Pankin et al., 2014). This gene is well adapted to the environments of China and Japan and is found in ICARDA/ CIMMYT genetic stocks (*CMB85533*, *Higuerilla*2/Gobernadora*) (Pankin et al., 2014). The amino-acid substitution in the GAF domain is the major influence of heading date under LDs (Nishida et al., 2013). However, *HvPHYC* may interact with other genes such as *Vrn-H1, sdw1* and *Ppd-H1* to induce early flowering under long and non-inductive short days (Hill et al., 2016; Nishida et al., 2013; Pankin et al., 2014). The QTL identified in this study behaved more like *HvPHYC* reported by Pankin et al. (2014) who observed 23 and 3 days differences in flowering between

the Bowman (late genotype) and Bowman (*eam5*) (early genotypes) under both short and long days, respectively.

However, with the existence of *Ppd-H1*, *eam5* showed no significant effect on maturity, i.e. no differences between Bowman *(Ppd-H1*) and Bowman (*Ppd-H1+eam5*) while the QTL identified in this study still showed earliness, indicating a possible new allele for early flowering.

3.4.2 Early heading affects some agronomic traits but not flour pasting properties

Previously reported *eps* QTL have been found to have direct influence on spike morphology, including length of the spike, spike density and thousand kernel weights in cereals (Faricelli et al., 2010; Gawroński et al., 2012; Hemming et al., 2009; Święcka et al., 2014; M. Zikhali & Griffiths, 2015) since the duration of vegetative period is positively correlated to spike length and spikelet number per spike (Kiss et al., 2014). NILs carrying the late alleles including the late parent Franklin were found to have longer spike length and higher grain number than those carrying the early alleles across all sowing dates (Table 3.2), due to prolonged duration of the spike developmental stages (Lewis et al., 2008; Święcka et al., 2014; M. Zikhali & Griffiths, 2015).

Ppd-H1 was reported to have *pleiotropic* effects on plant height (Laurie et al., 1994) and a QTL regulating heading date was found to be closely linked to the *sdw1* (*denso*) gene in barley (Barua et al., 1993). *Ppd-H1* also seems to be one of the key genetic determinants for plant height and tiller number with *Ppd-H1* reducing the number of tillers per plant (Alqudah et al., 2016). QTL regulating plant height in the DH population of TX9425 and Franklin are located on 2H and 3H (Wang et al., 2010), likely *uzu1* and *sdw1 (denso*), respectively. However, none

of these are in similar positions to early heading gene or are likely present in the NILs. Among four NIL pairs, 116 pair showed no significant difference in plant height, indicating no pleiotropic effect between the 5HL segment and plant height. The difference in the height between the alleles in the *317–1, 317–2 and 322* NIL pairs could be due to heading date or a different QTL responsible for plant height. Further studies are needed to confirm this.

Flour pasting properties are important quality traits and have close relationship with malting quality (Zhou et al., 2008; Zhou et al., 2005) and food processing quality (Zhou et al., 1998). Pasting properties have been found to be influenced by genotype (Zhou et al., 2008; Zhou et al., 1999) and environment (Wang. et al., 2010; Zhou et al., 1999; Zhou et al., 1998). *Earliness per se* can also influence grain protein content (Herndl et al., 2008) thus pasting properties (Zhou et al., 2000). Several QTL have been identified for pasting properties in barley (Wang. et al., 2010). These QTL for pasting properties are located on chromosomes 1H, 2H, 3H, 4H, 6H and 7H (Wang. et al., 2010) with no QTL on 5H, indicating that may be unlikely that this chromosome segment would affect pasting properties. Indeed, this was confirmed in the current experiment, with no significant differences measured between NILs with the early and the late alleles.

In conclusion, a QTL on chromosome 5HL that causes variations in heading/flowering date and growing degree-days to heading was identified from the cross between TX9425 and Franklin. Using different pairs of NILs, the gene was mapped to 122–129 cM with a large number of co-segregating markers. This locus was found to have less sensitivity to temperature and photoperiod compared with other maturity/vernalisation genes at similar positions, indicating a possible new allele for early flowering. The chromosome region results in significant effects on some agronomic traits such as the length of spike and the number of

spikelets per spike but has less effect on flour pasting properties. Thus, the locus showed no pleiotropic effects on grain pasting properties and other agronomic traits apart from those mentioned above. Since the maturity effects of *Eam5* are highly variable, closely linked molecular markers could be useful in facilitating the utilisation of this gene. These markers are much closer than previously reported *Raw1* locus, which is about 5 cm away from this earliness locus.

Chapter 4 Examining the yield potential of barley near-isogenic lines using a genotype by environment by management analysis

Abstract

This study modelled the value of adapting phenological traits of barley to suit diverse environmental and management conditions. Such trait manipulation will assist breeders in genotype selection and growers in better managing barley crops to achieve their yield potential. We first developed two near isogenic lines (NILs) of barley through perturbation of phenological genetics (*Eps-317-1-E*, and *Eps-317-1-L*). NILs were developed from a cross between TX9425, a Chinese landrace, and *Franklin*, an Australian cultivar. Field experiments were then conducted in Tasmania, Australia, using three sowing dates per year during 2015, 2016 and 2017 to parameterise and test the barley module of the APSIM model (*APSIM-Barley*). We then conducted a genotype by environment by management (GxExM) analysis using ten sites across the Australian wheat-belt, with a range of sowing dates, fertiliser rates and planting densities. The early genotype (*Eps-317-1-E*) performed better in environments prone to terminal drought and heat stress effects. This was due to earlier flowering and a propensity for greater transpiration-use efficiency from growth stage (GS) 50 to 87. The late NIL (*Eps-317-1-L*) generally produced higher yield in long-season environments with high rainfall and cool terminal temperatures. Performance of all genotypes was generally better for May sowings, wherein yields of the two NILs were highest. Overall, this study showed that *Eps-317-1-E* is more adapted to regions prone to drought and heat stress, while *Eps-317-1-L* is more suited to regions with longer growing seasons. This study exemplifies how models can be used in concert with breeding experiments, providing farmers and breeders with

opportunities to examine how new genotypes will perform in new environments under diverse management conditions.

4.1 Introduction

Barley (*Hordeum vulgare* L.) crops are cultivated globally for livestock and human consumption (FAO 2015), with the latter mostly used in the malting and brewing industries (Muñoz-Amatriaín et al., 2010). In Australia, 8,500 kt of barley was harvested in the 2016-17 growing season (ABARES 2017), illustrating the importance of this crop to the annual Australian cereal crop harvest.

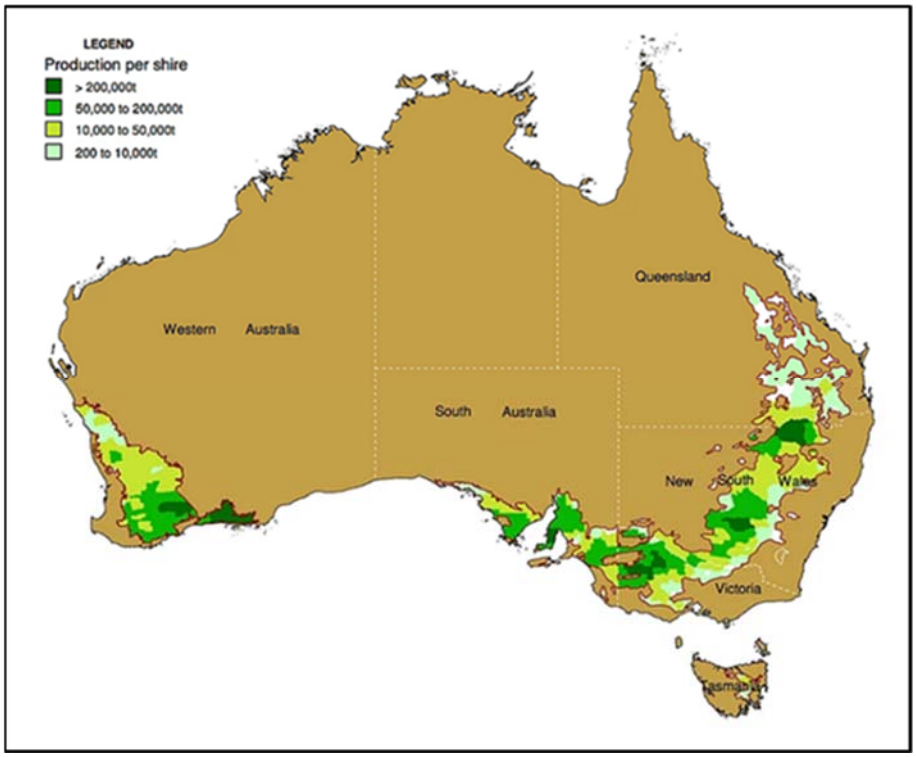

Figure 4.1 Australian barley production regions https://www.barleyaustralia.com.au/barley-and-malt. The site locations are listed on Table 4.2.

Barley production occurs throughout the mixed wheat-sheep region of Australia, with greatest annual average productivity in the South Australian and Victorian Mallee, central and northern NSW, and south-eastern WA (AEGIC 2017) (Fig. 4.1).

Global demand for this crop has increased from 133.2 million tonnes in 2000 to 146 million tonnes in 2017 without an obvious increase in production (IGC 2017), indicating an increasing supply/demand mismatch. Improving grain yield whilst maintaining grain quality to meet this demand in increasingly variable environmental conditions is a serious challenge facing barley breeders. Abiotic environmental stresses, such as waterlogging, drought, frost and heat events, are key factors that significantly affect barley growth and development and ultimately yield and quality.

Damage due to frost between spikelet initiation (Zadoks growth stage 30; GS30) to ear emergence (GS50) causes sterility of flowers (Al-Issawi et al., 2012) and results in yield loss amounting to $120 million per year in Australia (Barlow et al., 2013). At the other end of the growing season, drought and heat stress around anthesis (GS65) result in abortion and senescence of premature grains; indeed, in 2003 such abiotic stresses accounted for ~35% and ~75% yield reduction in barley and wheat, respectively (Barlow et al., 2013; Siebert et al., 2014). In order to constrain yield penalties under challenging climatic conditions, it is important to understand the mechanisms underpinning crop adaptation to local environments. The main factors influencing such adaptation include crop phenology, e.g., the timing and duration of reproductive stages, particularly that from spikelet initiation (GS30) to the end of grain filling (GS87) (Alvarez et al., 2016; Hill and Li 2016; Ibrahim et al., 2016). Matching the appearance of critical growth stages to the available environmental resources is paramount for achieving yield increase in new genotypes (Annicchiarico 2009; Ceccarelli et al., 2000; Chapman et al., 1993; Shorter et al., 1991), rather than just modifying crop agronomy alone (Ceccarelli et al., 2000).

To facilitate selection of genotypes for increased yield in different environments, conventional breeding experiments have separated the heading date of new varieties into early and late maturity (Nakamichi 2014). Further, plant breeding experiments are usually conducted at several locations over a limited number of seasons. In highly variable climates such as those in the wheat-belt of Australia (Meinke and Stone, 2005), the climatic conditions encountered during experimental trial periods are often not representative of the long-term climate conditions at these locations (Chapman et al., 2000). In other words, abiotic stress environment encountered during breeding trials will differ from season to season, which will bias the germplasm selection if that selection is based on a limited number of field evaluations. The resultant genotype by environment interactions (GxE) are often poorly understood and quantified. This reduces the power of the breeding process and makes the selection of superior genotypes for specific stress environments difficult (Rodriguez et al., 2008).

At the beginning of 21st century, genomics technology was used to identify, describe and map the major quantitative trait loci (QTL) and genes influencing phenology (Alvarez et al., 2016; Hill and Li 2016; Ibrahim et al., 2016). Of all the genes related to phenology, only two, photoperiod (*Ppd*), vernalisation (*Vrn*), have been cloned. Subsequently, genome wide association was used to link specific genomic regions with a phenotype (Hammer et al., 2016). These QTL or genes exhibit allelic, gene-gene and gene-environment interactions that cause variations in flowering time (Hammer et al., 2016; Hill and Li 2016; Yan et al., 2003). This further restricts our ability to understand the mechanisms regulating ear emergence (Hill and Li 2016; Ibrahim et al., 2016). In addition, complexities involved in transcriptions and post-transcriptions of genes compound the understanding and prediction of phenotype from the gene or QTL (Hammer et al., 2016), slowing progress in yield improvement in different

environment types (Hammer et al., 2016; Harrison et al., 2016). Usually, genes regulating late flowering may be desirable for a long season environment, while genes controlling earlier flowering (e.g. see Hill and Li 2016) may suit crops grown in shorter growing season environments in order to avoid late terminal stresses (Dofing 1999; Harrison et al., 2014), though such crop-climate interactions are difficult to foresee without use of a crop modelling framework.

Recent advances in understanding the productivity of barley genotypes under different environment types and crop management systems has involved exploiting the opportunities offered by genotype x environment x management interactions (GxExM or QTLxExM). This has already resulted in significant yield improvements in the US. For example, yield gain in maize over the years has been attributed to understanding GxM interactions by proactively adjusting management practices, such as changing sowing date, adjusting irrigation etc. (Duvick 2005). The use of GxExM analyses in conjunction with breeding programs to advance yield in barley is relatively premature, particularly studies of varied management conditions that may act symbiotically with yield progress of newly developed lines. Further, management decisions will depend on specific environmental conditions (Hammer and Jordan 2007; Harrison et al., 2014). For example, Hammer and Jordan (2007) showed that a sorghum genotype with high tillering potential was inappropriate for a double row-planting configuration in a drought prone environment, as this increased the demand for soil moisture. Whilst there has been considerable work on C_4 crops like maize and sorghum, there are very few GxExM analyses of barley, particularly those spanning the entire cropping zone of the Australian wheat belt.

Australia has diverse cropping environments and a highly variable climate. Hence, field trial evaluation of genotypes or near isogenic lines (NILs) consisting of a QTL or gene of interest for adaptation in several target population environments can be time consuming, laborious and prohibitively expensive. Instead, simulation models can be used to better understand the environmental challenges in adapting barley genotypes such that offspring have higher yield and grain quality under marginal- and climatically-variable land. Being a powerful process-based crop simulation model, the Agricultural Production Systems Simulator (APSIM) (Keating et al., 2003) can be used to effectively coalesce the primary factors affecting yield and quality in order to visualise the full potential of a genotype or QTL under different environmental stressors and management conditions (Hammer et al., 2016; Hammer and Jordan 2007). It follows that crop models such as APSIM-Barley can facilitate selection of appropriate genotype or QTL to specific environments. The model also describes and accounts for climate-plant-soil interactions that are emergent properties of GxExM analyses (APSIM 2015b).

Here we used a series of targeted breeding experiments to develop two new barley genotypes under field conditions in Tasmania, Australia. Although these genotypes share a similar genetic background, there is allelic variation within a region of the QTL at the interval of 122-130cM. The early and late genotypes developed are designated *Eps-317-1-E* and *Eps-317-1-L*, respectively (Ibrahim et al., 2018). We then conducted subsequent field experiments with these new genotypes in order to calibrate and validate APSIM. We then investigated the effects of environment and management on the performance of the new genotypes and parental lines in 10 barley-production regions of Australia using a GxExM analyses.

4.2 Materials and Methods

4.2.1 Field experiments

Field experiments were conducted under predominantly rain-fed conditions at the Tasmanian Institute of Agriculture, Mt Pleasant Laboratories at Prospect, Tasmania, Australia (41.4667^{o}S, 147.1500^{o}E). The site soil type is a sandy loam over clay with an elevation of 147 m above sea level. The site is exposed to a temperate maritime climate with a complex set of influences from bordering oceans (Lane et al., 2015; White et al., 2010).

4.2.1.1 Plant materials for the field experiment and data measurements

This study used two parental genotypes (Franklin and TX9425) and one pair of near isogenic lines (NILs). Franklin is a late maturing malting spring variety from Australia (DPIT 1989). TX9425 is also a spring variety but is a Chinese landrace that has relatively early heading and good waterlogging tolerance (Li et al., 2008; Zhou et al., 2007). The NIL pair includes *Eps-5HL-317-E1* and *Eps-5HL-317-L1* (hereafter, *Eps-317-1-E*, and *Eps-317-1-L* for the simplicity), and were derived from the cross between TX9425 and Franklin. The NILs have the same genetic background except for the QTL region carrying the gene controlling early (E) and late (L) maturity. All genotypes were sown in a randomised complete block design (RCBD) using three replications. Plots were 1200 mm x 1000 mm, with 250 mm between rows. Seed was sown at a depth of 25 mm in all years. Twenty seeds per row were sown, in each case achieving ~80 plants/m^2 at sowing. Fertiliser (150 kg/ha of 5:10:10:5 N:P:K:S) was applied at sowing while 100 kg urea/ha was applied at GS45. To determine the progress of spikelet initiation at GS30, barley primordia were dissected and examined using compound microscope (Leica DM 500, Leica Microsystems (Schweiz) AG, CH-9435 Heerbrugg, 2015) and microscope camera (Leica MC 170). Leaf area index (*LAI*) was measured using a canopy analyser system (SunScan SS-UM2.0 Delta Device Ltd. UK). Subsamples for determination of total aboveground biomass

(stems, leaves and spikes) per unit ground area were taken concurrently with *LAI* subsamples. Aboveground biomass was weighed before and after oven drying for three days at 85°C. Yield, plant height and yield components (number of spikelets per spike, spike length and 1000-kernel weight) were also determined at maturity.

4.2.2 Model parameterisation

APSIM version 7.4 (APSIM 2015b; Keating et al., 2003) was used to simulate barley growth and development. *APSIM-Barley* is structured around three major components; model engine, crop, surface organic matter and soil. The crop module in *APSIM-Barley* includes 11 different GS. These are determined by the accumulation of thermal time, which is influenced by photoperiod, vernalisation, nitrogen and availability of soil water (APSIM 2015b). The soil organic matter module accounts for both carbon and nitrogen dynamics in the soil. Soil organic matter is divided into three pools (fresh, biomass and humus), with the biomass pool representing the more labile, soil microbial biomass and microbial products. The simulated C and N ratios are indicative of the soil organic matter status, i.e. the labile carbon pool (Ahmed et al., 2016; APSIM 2015a). The model was parameterised according to APSIM guidelines (Table 4.1).

The model was calibrated following the procedure described by Gaydon et al. (2017) and Chen et al. (2010) using the measured data described above. Daily climate data including radiation, rainfall, maximum and minimum temperatures that were used in the model were obtained from the SILO database (http://www.longpaddock.qld.gov.au/silo) for Launceston, from 1 January 31 December 2015 (Fig. 4.2). Bulk density (BD) and volumetric water contents at air dry, lower limit (LL, i.e. 15 Bar), drained upper limit (DUL or field capacity) and saturation (SAT)

were determined from the Australian Soil Resource Information System (ASRIS 2018) (Table 4.1).

The parameterisation was conducted on 2015 and 2017 field data including phenology, LAI, biomass and grain yield, for the four genotypes; TX9425, Franklin, and the two NILs (*Eps-317-1-E* and *Eps-317-1-L*)

4.2.3 Model validation

Measurements of phenology, *LAI*, biomass and grain yield taken in 2016 were used for model validation. Model fitting evaluation statistics included the Pearson correlation coefficient (r), coefficient of determination (R^2), mean prediction error (MPE), modelling efficiency (MEF), mean bias (MB), concordance correlation coefficient (CCC) (Pembleton et al., 2015; Tedeschi 2006). The R^2 and r statistics are indicators of precision (Tedeschi 2006), with values closer to 1.0 indicating better quality of fit (Gaydon et al., 2017; Tedeschi 2006). The CCC and MEF also are measures of precision and accuracy; ideally the CCC will be 1.0 (lower values indicate bias from the 1:1 line and less than ideal fit) and ideally the MEF will be greater than 0.5; if the MEF is less than zero, the modelled values are worse than the observed mean. The MB statistic is computed as the mean difference between the observed and simulated mean values. Values higher than 0.5 are desirable, with values of 1.0 indicating ideal fit (Pembleton et al., 2015; Tedeschi 2006).

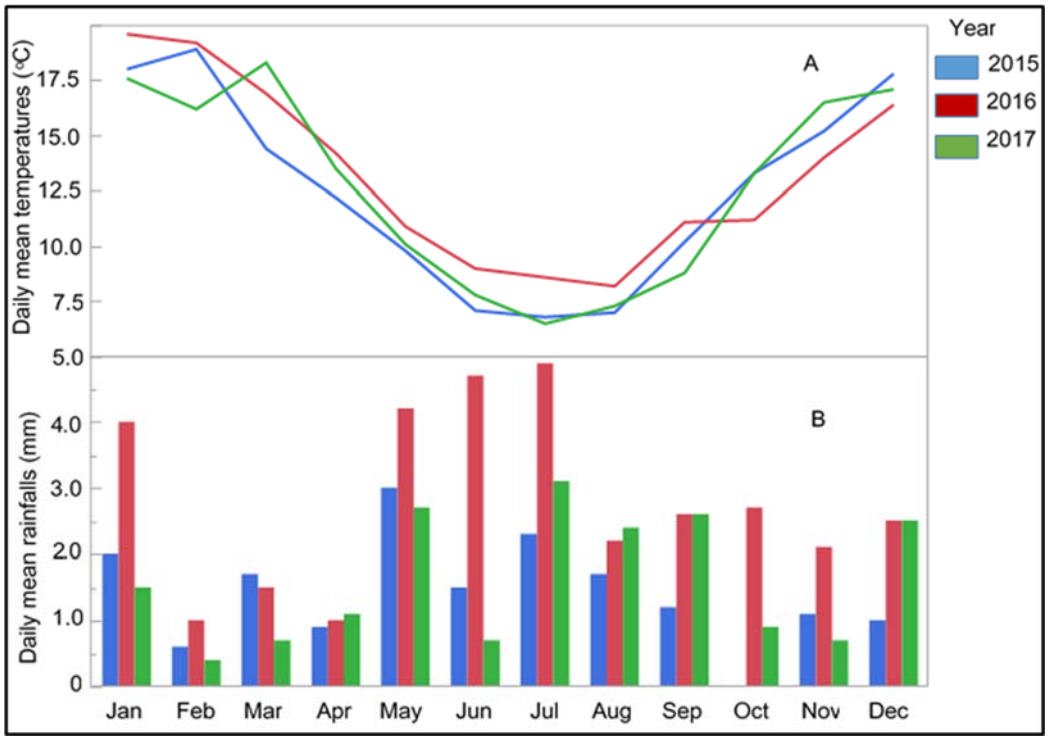

Figure 4.2 Daily mean of temperatures and (B) rainfall distributions during the years 2015, 2016 and 2017.

4.2.4 Simulation of genotype by environment by management interactions

Six agro-ecological regions classified by Barley Australia (BA) and the Australian Export Grains Innovation Centre (AEGIC; *https://www.barleyaustralia.com.au/barley-and-malt*) were used to select 10 sites for this study (Fig. 4.1).

Two contrasting sites from each region (shire) were selected (Table 4.2). The combination of varied soil characteristics and climatic conditions constituted a representative population of barley cropping environments that was necessary for this study. Daily climate data was obtained as a SILO patch-point data set and was used to conduct the GxExM simulations using the parameterised *APSIM-Barley* model.

Table 4.1 Soil physical characteristics of the field site, including bulk density (BD) and water contents at air dry, crop lower limit (LL15), drained upper limit (DUL) saturation (SAT), hydraulic conductivity (KS), soil pH (water) and soil organic carbon (OC).

Depth (cm)	Description	BD (g/cc)	Air dry (mm/mm)	LL15 (mm/mm)	DUL (mm/mm)	SAT (mm/mm)	KS (mm/day)	PH (-)	OC (%)
0-15	**Clay sand**	1.0	0.297	0.330	0.478	0.503	288.0	7.0	1.0
15-30	**Sandy clay loam + (A2 layer (highly leached)**	1.2	0.315	0.350	0.450	0.517	144.0	7.0	0.5
30-60	**Medium clay + A2 layer**	1.2	0.360	0.400	0.490	0.517	72.0	6.9	0.25
60-90	**Clay**	1.3	0.360	0.400	0.500	0.517	72.0	6.9	0.25

Table 4.2 Site descriptions including climate and soil characteristics, as well as long-term productivity per region.

Regions	Site	Latitude and Longitude (°N, °E)	Annual average temperature (°C)	Annual rainfall range (mm)	APSIM soil number	Prod./region (tonnes)
Tasmania	Northdown	-41.18, 146.48	13	540-1300	775	200-10,000
QLD	Capella	-23.09, 148.02	23	190-1140	049	200-10,000
New South Wales	Pallamallawa	-29.47, 150.14	19	262-1019	055	>200,000
	Grenfell	-33.95, 148.08	15	451-1028	547	200-10,000
Southern Australia	Ardrossan	-34.42, 137.92	17	178-605	257	>200,000
	Denial bay	-32.10, 133.58	18	216-407	328	200-10,000
Victoria	Yarrawonga	-36.03, 146.00	16	212-880	629	10,000-50,000
	Lexton	-37.27, 143.52	14	260-913	710	10,000-50,000
Western Australia:	Esperance	-33.85, 121.88	17	425-903	453	200-10,000
	Binnu	-28.04, 114.68	20	162-578	491	>200,000

Sources for meteorological and production data: APSIM Soil, APSoil database (https://www.apsim.info/Products/APSoil.aspx, https://www.barleyaustralia.com.au/barley-and-malt?lightbox=dataItem-imlgggqs, http://www.bom.gov.au/climate/data/).

4.2.5 Crop management

Management (M) factors included sowing dates (SD), planting density (PD) and nitrogen fertilisation level at sowing (N) (Table 4.3). Selection of the sowing dates was such that the genotypes were simulated under a range of photoperiods, vernalisation periods and temperatures at each site.

Table 4.3 Management practices (M) involving sowing date, plant density, and nitrogen levels used for the GxExM factorial simulations.

Management	**Levels**			
Sowing date	1 March	1 April	1 May	1 June
Plant density (plants/m^2)	60	80	100	120
Nitrogen levels (kg/ha)	0	50	100	150

4.2.6 Statistical analyses

Simulated phenology, grain yield and protein content for the four genotypes were analysed statistically as a combined analysis of variance involving ten sites, twenty years, four sowing dates, four nitrogen (N) levels and three planting densities following the approach described by Singh and Chaudhary (1979). We employed the generalised linear model (GLM) SAS/JMP 13.0 to run the statistical analyses, assuming significance at the 0.05 level of probability. Post-hoc Tukey's Honest Significant Difference tests were used to rank means.

4.3 Results

4.3.1 Validation

4.3.1.1 Validation of phenology simulations

Mean phenological values of the four genotypes were similar, with simulated values of 51, 51, 52 and 51 for TX9425, Franklin, *Eps-317-1-E* and *Eps-317-1-L*, respectively. Observed floral initiation (FI) occurred 5 days later than the simulated values for TX9425 and Franklin, while

Eps-317-1-E and *Eps-317-1-L* each had identical observed and simulated FI dates, indicating good calibration. Observed and simulated heading date occurred on the same day for TX9425, whilst observed heading date was 1, 2 and 5 days later than the simulated dates for Franklin, *Eps-317-1-E and Eps-317-1-L*, respectively. The model therefore predicted the phenology adequately, with R^2 values close to 1.0 for all genotypes (Table 4.4). The MPE was very good to excellent (usually less than 5%) and the modelling efficiency was very good (0.5 < MEF < 1). The BCF and CCC were 1.0 for all genotypes, indicating that there was no bias from the 1:1 line fitted to observed versus predicted values

4.3.1.2 Validation of leaf area index simulations

There was very good agreement between the simulated and observed LAI for all the genotypes with R^2 and r ranges from 0.9-1.0 (Table 4.5). The MB indicated minor differences between modelled and measured mean values, with values ranging from -0.01 to 0.04. This was further confirmed by the CCC value, which indicated only a slight bias from the 1:1 model-prediction line. The mean LAI was highest for *Eps-317-1-L* and TX9425 with both close to 1.2, while *Eps-317-1-E* and *Franklin* had a similar value (0.7).

4.3.1.3 Validation of biomass simulations

The goodness of fit of the model for the biomass simulations demonstrated excellent validation, evidenced by an R^2 value of 1.0 (Table 4.6). Although TX9425 and Eps-317-E were slightly over-estimated (MB > 1), Franklin was better predicted with the lowest MPE value 1.8%.

Table 4.4 Validation statistics of APSIM-Barley simulations; data shown include mean observed and simulated phenology for the four barley genotypes, TX9425, Franklin, Eps-317-1-E (early) and Eps-317-1-L (late).

Variable	TX9425	Franklin	Eps-317-1-E	Eps-317-1-L
Observed	51	51	51	51
Simulated	51	51	52	51
R^2 [a]	1.0	1.0	1.0	1.0
r[b]	1.0	1.0	1.0	1.0
MB[c]	-0.0	0.0	-1.1	-0.3
MPE[d]	4%	6%	5%	5%
MEF[e]	1.0	1.0	1.0	1.0
VR[f]	1.0	1.0	1.0	1.0
BCF[g]	1.0	1.0	1.0	1.0
CCC[h]	1.0	1.0	1.0	1.0
CCC[h]	1.0	1.0	1.0	1.0

[a]Coefficient of determination (R^2) with perfect agreement at 1 or 100%
[b]Pearson correlation coefficient (r) with perfect agreement at 1
[c]Mean bias (MB). Ideal = 0. MB < 1 represents under estimation and MB > 1 represents over estimation of the modelled data
[d]Mean prediction error (MPE) <5% = Excellent, 5-10% = Very good, 10-20% = moderate, >20% = poor
[e]Modelling efficiency (MEF) ideally higher than 0.50, if MEF is lower than zero, the model-predicted values are worse than the observed mean
[e]Variance ratio. Ideal = 1, greater than 1 = more variation in actual than simulated data
[g]Bias correction factor. Ideal = 1, lower values indicate bias from the 1:1 line
[h]Concordance correlation coefficient (CCC). Ideal = 1, lower values indicate bias from the 1:1 line and a less than ideal fit

Table 4.5 Validation statistics of APSIM-Barley simulations of leaf area index (LAI) for four barley cultivars; TX9425, Franklin, Eps-317-1-E (early) and Eps-317-1-L (late). Abbreviations in the first column are as per footnotes in Table 4.4.

Variable	TX9425	Franklin	Eps-317-1-E	Eps-317-1-L
Obs	1.2	0.7	0.7	1.2
Sim	1.3	0.7	0.7	1.2
R^2	1.0	0.9	1.0	1.0
R	1.0	1.0	1.0	1.0
MB	-0.1	0.0	0.0	0.0
MPE	17%	34%	16%	9%
MEF	1.0	1.0	1.0	1.0
VR	1.0	1.1	1.2	1.0
BCF	1.0	1.0	1.0	1.0
CCC	1.0	1.0	1.0	1.0

Table 4.6 Validation statistics of APSIM-Barley performance for biomass simulations for four barley cultivars; TX9425, Franklin, Eps-317-1-E (early) and Eps-317-1-L (late). Abbreviations in the first column are as per footnotes in Table 4.4.

Variable	TX9425	Franklin	Eps-317-1-E	Eps-317-1-L
Obs	364	209	196	315
Sim	377	208	204	300
R^2	1.0	1.0	1.0	1.0
R	1.0	1.0	1.0	1.0
MB	11	1	8	145
MPE	6%	2%	61%	16%
MEF	1.0	1.0	1.0	1.0
VR	1.0	1.1	1.2	1.0
BCF	1.0	1.0	1.0	1.0
CCC	1.0	1.0	1.0	1.0

4.3.2 Statistical analysis of the GxExM

An analysis of variance showed that genotype (G) accounted for 39%, 90% and 73% of simulated variation in floral initiation, grain size and grain protein, respectively (Table 4.7). TX9425 and *Eps-317-1-E* were the earliest to head (91 and 100 DAS respectively) and had the lowest dry matter (5.6 and 8.0 t/ha respectively) and grain yield (1.5 and 2.2 t/ha respectively). Both *Eps-317-1-L* and Franklin were later heading and had greater grain yield across all of the environments

The environment component (consisting of both soil and climate) were highly variable for all traits (Table 4. 7). The effects were larger on heading date, maturity date, shoot biomass, and grain yield, accounting for 49%, 50%, 41% and 45%, respectively (Table 4.7). Floral initiation date ranged between 42-88 DAS and heading date ranged between 69-135 DAS. Genotypes in Capella (QLD) and Binnu (WA) developed quicker than crops at other sites, with heading dates of 69 and 83 DAS, respectively, while genotypes developed much later in Northdown (TAS), Lexton (VIC) and Grenfell (NSW) (Fig. 4.3).

For the interactions, (genotype x site x sowing date, GxSxSD) was responsible for most of the variation for all traits (Table 4.7). Genotype x site x year (GxSxY) had strong effects on maturity date, biomass, grain size, grain protein and yield, but had little effects on flower initiation date and heading date. Similarly, significant differences were obtained for biomass, grain size, grain protein and grain yield due to genotype x site x nitrogen level (GxSxN) effects. Genotype x environment plant density (GxExPD) only had major effects on biomass and grain yield.

Table 4.7 Analysis of variance (ANOVA) of the genotype, management and genotype by environment by management interactions (GxExM) for all traits.

Source of variance	DF	FI[a]	HD[b]	MAT[c]	BM[d]	GS[e]	GP[f]	GY[g]
Genotype (G)	3	1247510*	1069199*	2550489*	22399*	3*	141028*	13954407*
Environment (E)								
Site (S)	9	1014518*	1999502*	3085655*	39710*	0.21*	16734*	31590703*
Year (Y)	20	16632*	23342*	23008*	18600*	0.08*	896*	16500000*
Management (M)								
Sowing date (SD)	3	9446228*	1056191*	597805*	6539*	0.07*	291*	6054096*
Nitrogen level (N)	3	119	86	87	49553**	0*	33118*	33044894*
Plant density (PD)	2	0.4	0.7	1.3	1936*	0*	181*	335674*
GxExM								
G*S*SD	81	727*	1127*	3326*	2353*	0*	80*	34895*
G*S*Y	450	134	139	571*	29*	0*	77*	4692404*
G*S*N	81	48	44	91	62*	0*	126*	62758*
G*S*PD	54	2	2	6	6*	0	9	6279*

Levels of significance: * Highly significant ($p < 0.001$). [a]Floral initiation date (DAS), [b]Heading date (DAS), [c]Maturity date (DAS), [d]Biomass (t/ha), [e]Grain size (mm), [f]Grain protein (%) and [g]Grain yield (t/ha).

Table 4.8 Proportions of the total variance (%) for the genotype, environment and management in the ANOVA for each trait. Abbreviations as per Table 4.7.

Sources of variance	FI[a]	HD[b]	MAT[c]	BM[d]	GS[e]	GP[f]	GY[g]
Genotype	39	26	41	16	90	73	13
Environment (Site+year)	32	49	50	41	8	9	45
Management	29	26	10	41	2	18	37

[a]Floral initiation date (DAS), [b]Heading date (DAS), [c]Maturity date (DAS), [d]Biomass (t/ha), [e]Grain size (mm), [f]Grain protein (%) and [g]Grain yield (t/ha).

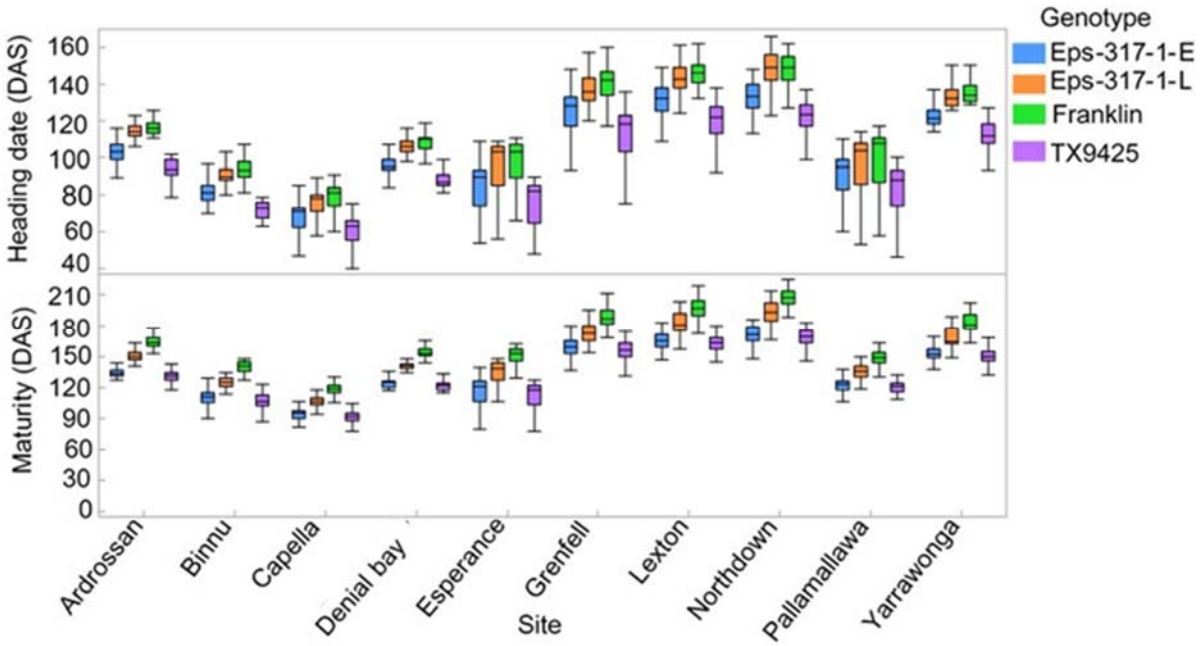

Figure 4.3 Variation in heading date and maturity of four barley genotypes used for the APSIM-Barley GxExM simulations across ten sites in Australia (DAS = days after sowing). Simulations were performed from 1997 to 2017 using multiple levels of three management factors (see table 4.3).

Although Year had a strong influence on all traits across genotypes, this was likely due to the high degrees of freedom of this variable (Table 4.7). Grain protein ranged from 7% in 2016 to 10% in 1997, while grain yield ranged from 1.7 to 2.8 tonnes/ha, with the highest yield in 1997

and lowest in 2013, 2014 and 2016. This result can be interpreted by examining the simulated stress factors. In 1997, TX9425 had lower access to nitrogen at the onset of grain filling period, which affected leaf expansion, photosynthesis and grain filling (Fig. 4.4). In 2016, nitrogen limitation occurred at GS 31, much earlier than in 1997. Similarly, *Eps-317-1-E* experienced more severe N stress in 2016 compared with 1997 (Fig. 4.5). The late genotypes, Franklin and *Eps-317-1-L*, were affected by similar stress factors, predominantly N limitation of photosynthesis and leaf expansion (Fig. 4.6 and Fig. 4.7).

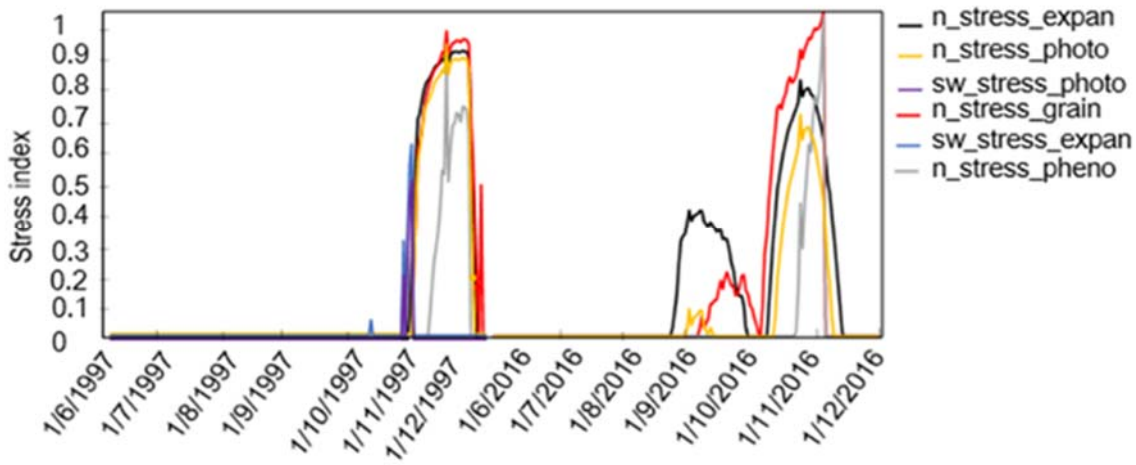

Figure 4.4 Simulated crop nitrogen stresses: n_stress_expan, n_stress_photo, n_stress_grain and n_stress_pheno as nitrogen stresses for leaf expansion, photosynthesis, grain filling and phenology respectively. Soil water stresses (sw_stress_photo and sw_stress_photo and sw_stress_expan) represent photosynthesis and leaf area expansion respectively. Simulation was conducted for Denial-Bay in 1997 and 2016 for TX9425.

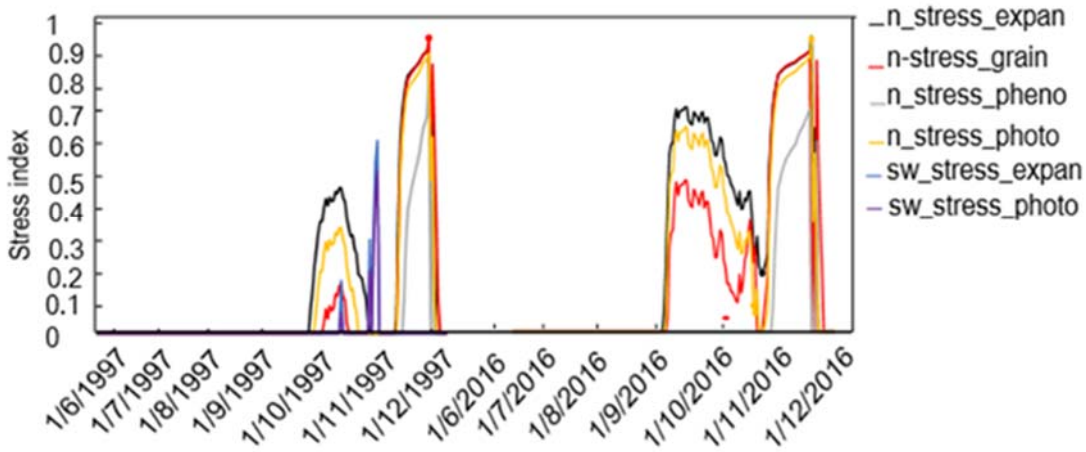

Figure 4.5 Simulated crop nitrogen stresses: n_stress_expan, n_stress_grain, n_stress_pheno, and n_stress_photo, as nitrogen stresses for leaf expansion, grain filling, phenology and photosynthesis respectively. Soil water stresses (sw_stress_expan and sw_stress_photo) represent leaf area expansion and photosynthesis respectively. Simulation was conducted in Denial-Bay in 1997 and 2016 for Eps-317-1-E.

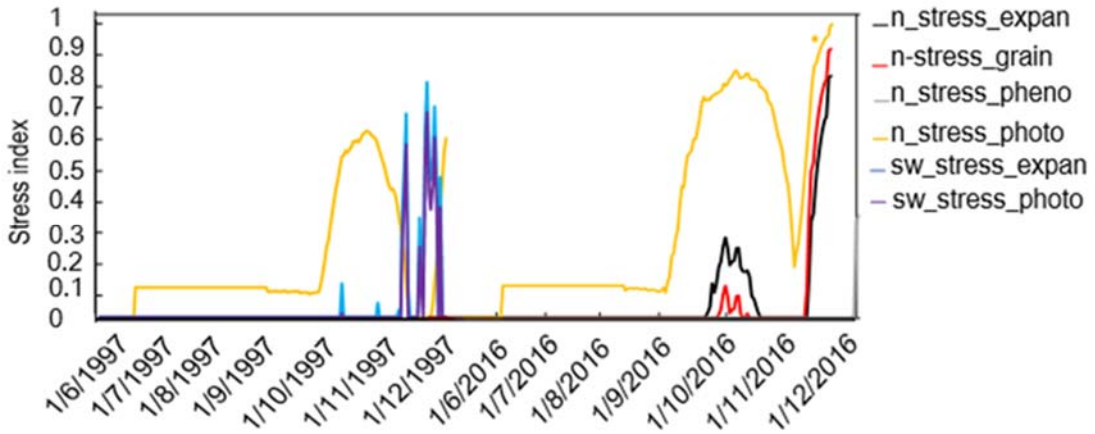

Figure 4.6 Simulated crop nitrogen stresses: n_stress_expan, n_stress_grain, n_stress_pheno, and n_stress_photo, as nitrogen stresses for leaf expansion, grain filling, phenology and photosynthesis respectively. Soli water stresses; sw_stress_expan and sw_stress_photo: leaf area expansion and photosynthesis respectively in Denial-Bay in 1997 and 2016 for Franklin.

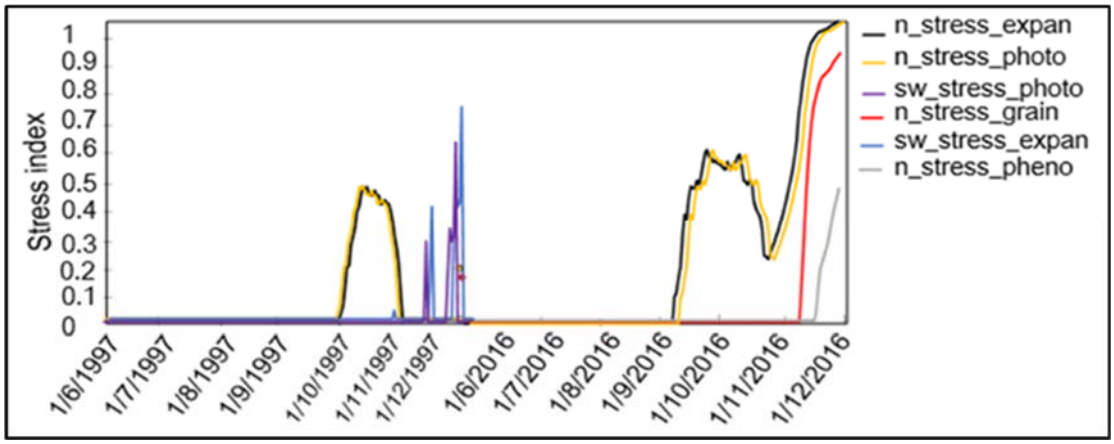

Figure 4.7 Simulated crop nitrogen stresses n_stress_expan, n_stress_photo, n_stress_grain and n_stress_pheno as nitrogen stresses for leaf expansion, photosynthesis, grain filling and phenology respectively. Soil water stresses (sw_stress_photo and sw_stress_expan) photosynthesis and leaf area expansion respectively. Simulation was conducted for Denial-Bay in 1997 and 2016 for Eps317-1-L.

4.3.3 Variation in phenology and yield across environments

Fig. 4.3 demonstrates significant variation across sites for heading and maturity date accounting for larger proportion of the variation (table 8). The sites of Capella and Binnu had the earliest heading and maturity date for the genotypes, whereas Northdown and Lexton had the longest growing seasons. TX9425 was consistently the earliest in all sites followed by *Eps-317-1-E*, while *Franklin* and *Eps-317-1-L* were later at all sites (Fig. 4.3). On average, *Franklin* required 114 DAS to heading and matured at 164 DAS, while *Eps-317-1-L* required 110 DAS to heading and matured at 145 DAS. Biomass and grain yield were highly variable among genotypes within and across the sites and years (Fig. 4.8 and 4.9). TX9425 consistently had the lowest yield across sites compared to other genotypes. *Franklin* had the highest biomass, but not always the highest grain yield; indeed, in many cases, *Eps-317-1-L* had the greatest grain yield (Fig. 4.8).

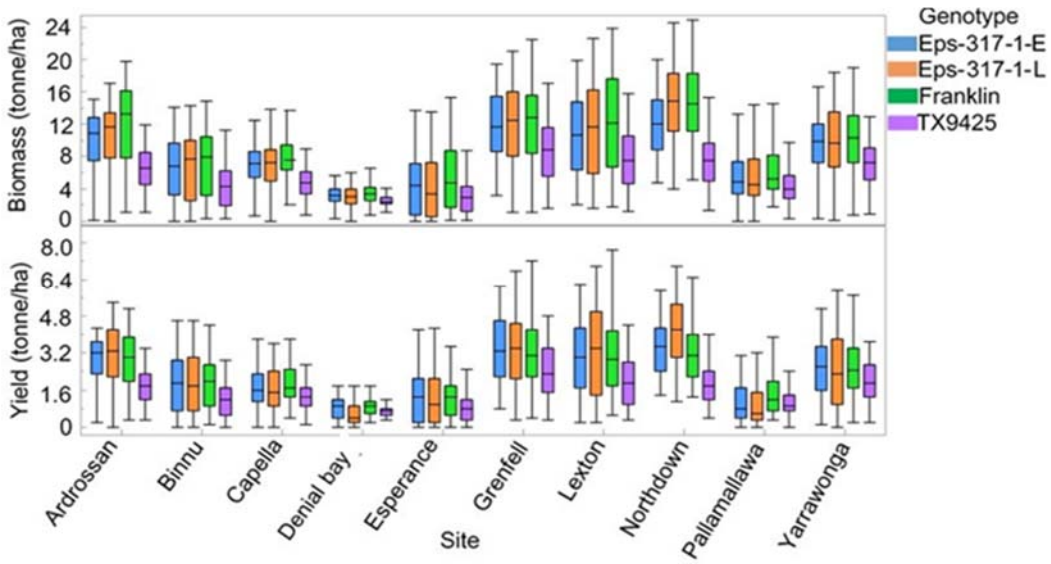

Figure 4.8 Annual variation in biomass and grain yield of four barley genotypes used for the APSIM-Barley GxExM simulations across ten sites in Australia. Simulations were performed from 1997 to 2017 using multiple levels of three management factors (see Table 4.3).

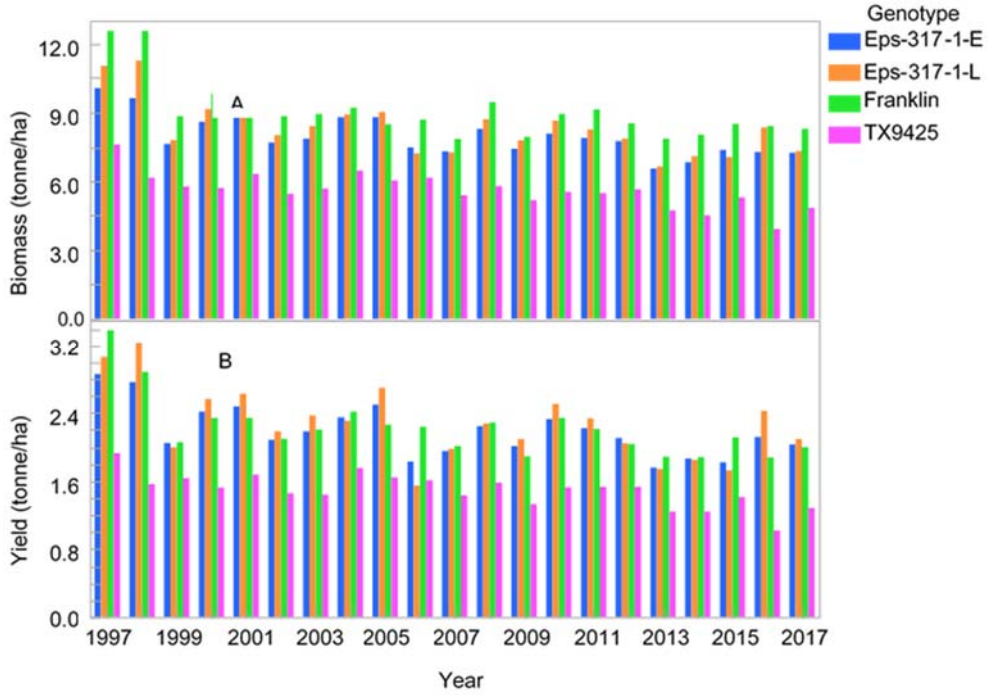

Figure 4.9 Annual variation in biomass (A) and grain yield (B) of four barley genotypes used for GxExM simulations from 1997 to 2017 across ten sites in Australia. Simulations were performed from 1997 to 2017 using multiple levels of three management factors (see Table 4.3).

However, Eps-317-1-E had better yield compared with other genotypes in Binnu, Capella, Denial Bay, Esperance and Yarrawonga. All genotypes performed very poorly in Denial Bay due to high terminal temperatures coupled with low growing season rainfall. The biomass and yield of all the genotypes were higher in 1997 and 1998 compared to the rest of the years (Fig. 4.9 and 4.10) partly due to higher rain falls in those years (data not sown).

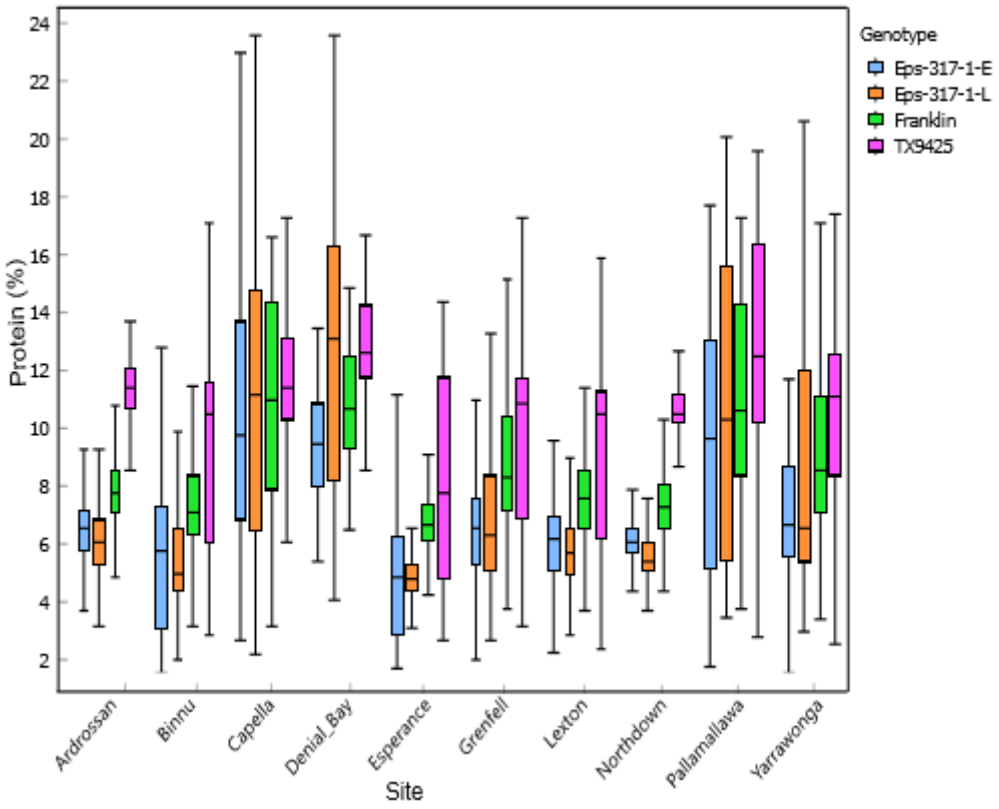

Figure 4.10 Long-term inter-annual variation in grain protein of four barley genotypes used in GxExM simulations across ten different sites in Australia

4.3.4 Effects of management on genotypic performance

Sowing time had significant effects on heading and maturity date in all genotypes (Fig. 4.11). TX9425 headed earlier than the other genotypes across the sowing dates, followed by *Eps-317-1-E.* All genotypes matured earlier when sown in March compared with the April sowing,

while there was no significant difference in the heading date between *Eps-317-1-L* and *Franklin* when sown in April, May and June. Nitrogen fertiliser and plant density had no effect on heading or maturity date.

Biomass and grain yield were higher when the genotypes were sown in May compared with other sowing dates except for TX9425, which had higher yield when sown in June (Fig. 4.12). March-sown plants generally had the lowest grain yield values compared with other sowing dates.

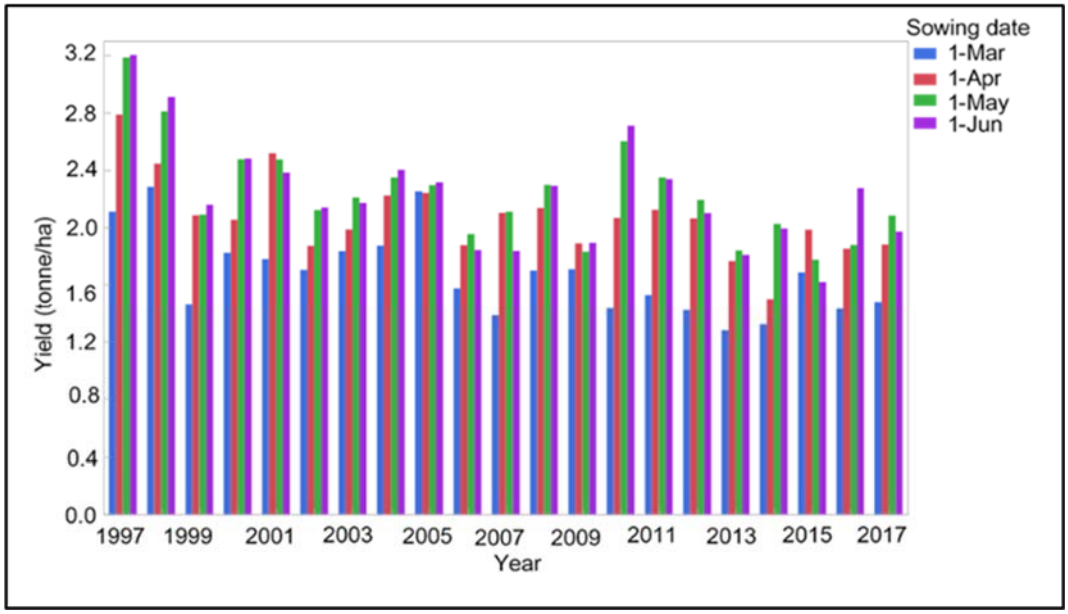

Figure 4.11 Simulated grain yield of all the genotypes from 1997 to 2017 across the ten environments under various sowing dates. Each bar plot contains all factors other than that shown on the horizontal axis

Grain yield increased with increasing N fertiliser up to 100 kg N/ha in all the genotypes (Fig. 4.12). There were only minor yield differences between *Eps-317-1-E, Eps-317-1-L* and Franklin when 50 kg of N fertiliser was applied per hectare.

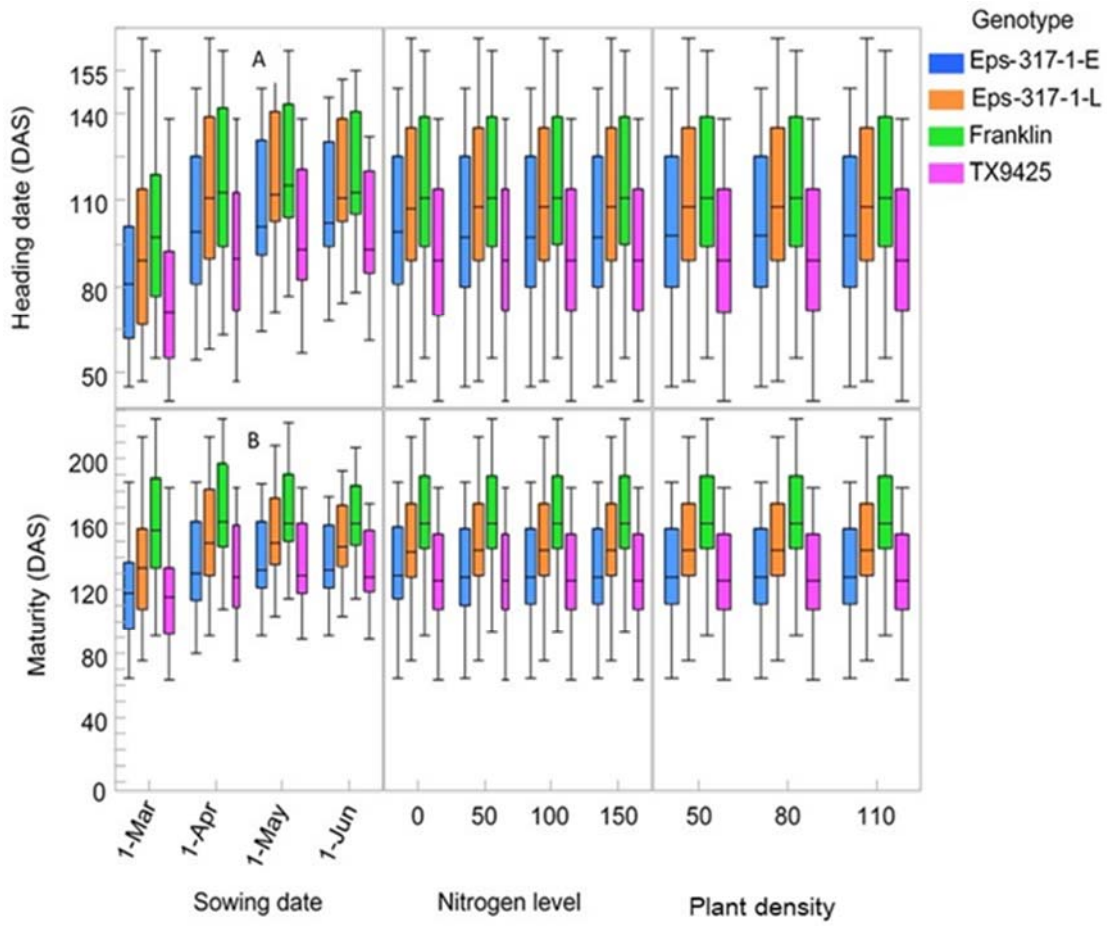

Figure 4.12 Heading and maturity days after sowing of four barley genotypes due to different crop management, sowing date, nitrogen levels and plant density. Nitrogen levels are given in kg/ha and plant density in plants/m2. Each boxplot contains all factors shown in Table 4.2 other than that shown on the horizontal axis.

Changes in plant densities did not significantly affect the yield in any of the genotypes, although TX9425 exhibited a moderate increase in median yield and yield variability with increasing planting densities (Fig. 4.12) and consistently had higher grain protein (Fig. 4.13). TX9425 also performed best in Yarrawonga when sown in April and June and Pallamallawa when sown in May and June (data not shown). *Eps-317-1-L* had yield advantage in Ardrossan when planted in March, April, and May. This genotype also had better yield in Northdown and Grenfell for all sowing dates simulated here (data not shown).

Furthermore, TX9425 had higher grain protein at Binnu, Esperance, Lexton, Ardossan, Denial Bay and Capella, especially when sown in April and March. This was followed by *Eps-317-1-L* at Denial Bay for the May and June sowing dates. *Eps-317-1-L* had higher grain protein in Capella for the March sowing and Pallamallawa for the June sowing (data not shown).

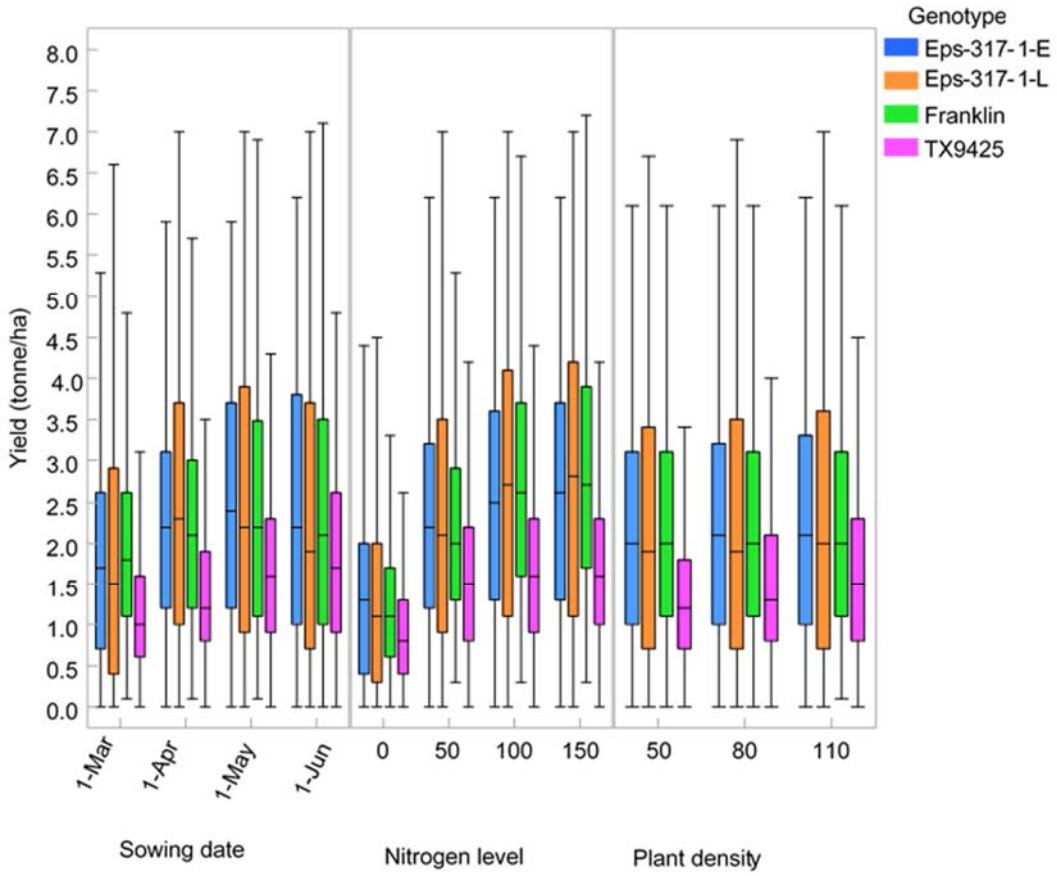

Figure 4.13 Annual average grain yield of four barley genotypes subjected to a range of sowing dates, nitrogen fertiliser rates (kg N/ha) and planting densities (plants/m2). Each boxplot contains all factors in Table 4.2 other than that shown on the horizontal axis.

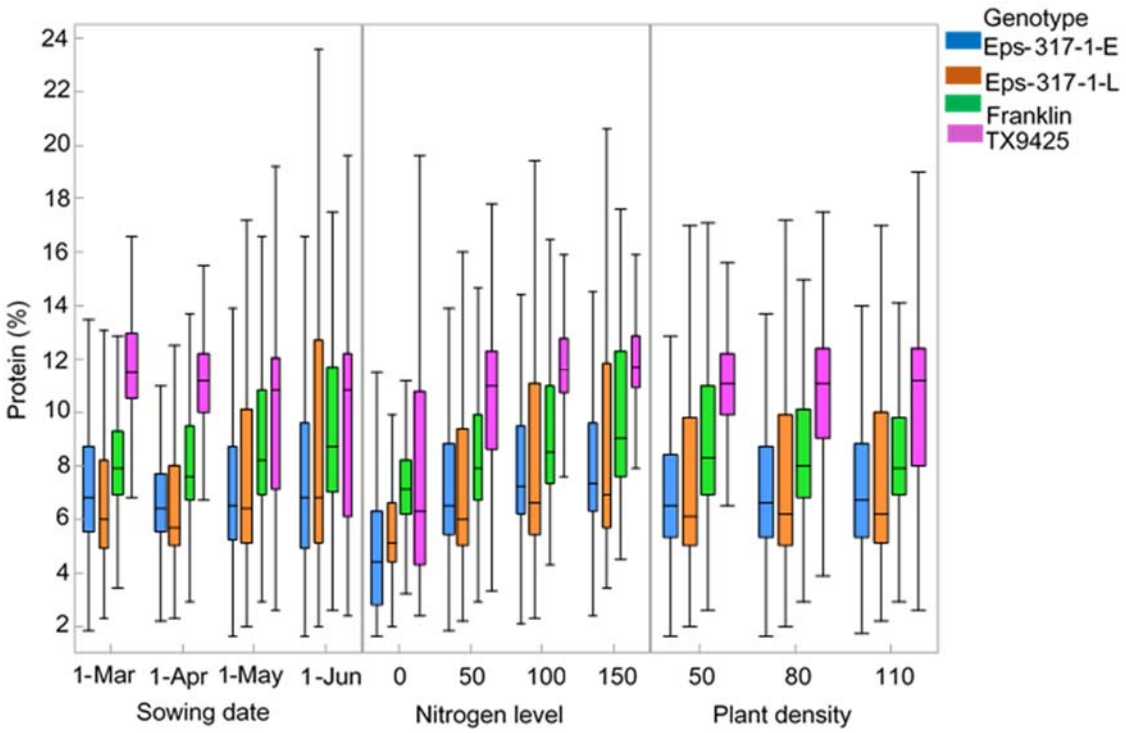

Figure 4.14 Grain protein of four barley genotypes in response to different crop management, sowing date, nitrogen levels and plant density. Each boxplot contains all factors in Table 4.2 other than that shown on the horizontal axis.

4.4 Discussion

This study used APSIM-Barley to understand differences among four barley genotypes caused by phenology across a range of environmental conditions and management practices. The simulations conducted in the present study suggest that in order to obtain optimum yield, consideration of the specific environment and management practices are important for all genotypes, particularly new breeding lines (Table 4.7).

When pooled across all environments (site and years), the late heading genotype containing the late allele (*Eps-317-1-L*) had higher grain yield compared with *Franklin* and early allele (*Eps-317-1-E*) (e.g. Fig. 4.12). This was because the late allele (*Eps-317-1-L*) induces and produced more tillers, resulting in greater biomass under adequate soil water and optimum temperature compared with other genotypes (data not shown). *Franklin* generally yielded second highest, even though it typically had the highest biomass (Fig. 4.9). Greater biomass accumulation by *Franklin* may have resulted in faster depletion of soil water and nitrogen and thus earlier onset of N stress, affecting leaf expansion, canopy photosynthesis and the extent of grain filling, particularly in dry years such as 2016 (Fig. 4.6 and 4.11). Results from wheat experiments showed that low soil nitrogen during early phenological stages inhibits leaf expansion, affecting the photosynthetic rates and therefore the amount of photosynthate available for grain filling (Meinke et al., 1997). Our results confirm these findings for all genotypes, and TX9425 in particular. During heading and anthesis, about 10-60% yield loss can be attributed to nitrogen stress in crops when soil water is adequate (Sadras et al., 2016). This indicates that the physiological outcome of the stress depends on its severity and the growth stage of the genotype, with the flowering window being a crucial period for farmers to minimise stresses in order to maximise yield potential (Damisch and Wiberg 1991). For the

grain protein (GP) and size (GS), all the components (genotype, environment and management) were found to influence the two traits in which (genetic factor) genotypic differences (Table 4.8) accounted for the larger proportion of the variance (Studnicki et al 2016). TX9425, with smallest GS (data not shown) consistently has the highest grain protein content among the genotypes followed by Franklin across the environments and managements practices (Fig 10 and 14). This may have been due to the earliness and lower yield of the TX9425 indicating a negative correlation between GP and yield (Studnicki et al 2016). While the high rate tillers producing Franklin might have rapidly depleted the available plant resources before reaching the grain filling period. Plants that experience stress especially before or during the grain filling period usually have higher grain protein and proline accumulation (Ahmed and Hassan 2015)

4.4.1 Response of new and existing barley genotypes to different environments

Table 4.7 indicates that environment had significant effects on FI, HD, GP and GY. This result underscores the need to account for environment in selecting appropriate genes for target populations of environments during crop improvement (Chapman et al., 2000; Chapman et al., 2002). In our experiments, cultivars required ~135 days to head in Northdown compared with ~69 days at Capella. Earlier phenological development in Capella, Binnu and Pallamallawa was mostly due to the greater rate of degree-day accumulation in these locations. Higher temperatures over the growing season generally can cause earlier expression of flowering genes, and this can be exacerbated by other soil-related stress factors depending on cultivar sensitivity (Slafer and Rawson 1995). Riazuddin et al. (2010) stated that an increase in mean daily temperature could cause earlier maturity, which may reduce yield if conditions permitted longer growing seasons. The combination of low rainfall and relatively

high temperature at these sites hastened water loss through evapotranspiration. Other studies have also suggested that high temperatures at anthesis have negative effects on yield due to increased pollen sterility, reduced ovule viability and spikelet abortion (Hasanuzzaman et al., 2013). In addition, high temperature can reduce the solubility of the oxygen, thereby increasing photorespiration and reducing photosynthesis (Prasad et al., 2008), and oxidative stress arising from increased reactive oxygen species reduces the rate of grain development (Hasanuzzaman et al., 2013; Suriyasak et al., 2017).

4.4.2 Response of new and existing barley genotypes to management

The divergence in heading date and yield underscores strong effects of the management in breeding programs. Hence, to maximise yield, optimising sowing times for each genotype should be aimed for so that heading can occur during lowest long-term frequencies of frost and drought. Figs 4.11 and 4.12 showed that sowing in May and June generally had similar effects on heading date and resulted in higher yield, because plants have optimum temperature and adequate moisture for growth and development over the long-term. On the other hand, simulations in which sowing was performed earlier (e.g. March), the late allele genotypes (*Eps-317-1-L*) and *Franklin* often experienced soil water and nitrogen stress in April sowing compared with other genotypes (data not shown), which may have hastened flowering and truncated later potential biomass accumulation.

Our study also showed little effect of N fertilisation on phenology, which was in line with results reported by McGuire et al. (1979). Mesfin and Zemach (2015) observed similar results where nitrogen fertiliser had no significant effect on days to heading in barley.

Yield increases with increasing nitrogen level under water non-limiting conditions were likely due to higher biomass accumulation, including higher number of tillers and taller plants. One of the most important effects of increased nitrogen levels is its effects on leaf expansion, and consequently leaf area index. A higher the leaf area index (within limits) generally leads to an increase in light interception, which in turn increases plant growth, biomass and grain yield (Meinke et al., 1997).

4.4.3 Breeding for crop adaptation

Increasing demand for barley has led breeders to attempt to genetically improve yield through matching genotype and management to specific environments (specific adaptation). In this simulation, both early and late alleles and cultivars responded differently across sites. For instance, when looking at the comparative yield advantage, TX9425 was the earliest to mature but had the lowest yield across environments except Denial Bay and Pallamallawa (Fig. 4.11), while genotype carrying the early allele (*Eps-317-1-E*) was generally more moderately to broadly adapt to environments and management practices examined here. The success of this allele, *Eps-317-1-E,* could be attributed to its later heading date but similar maturity date compared with *TX9425* cultivar, a faster growth rate and relative smaller mean of leaf area index that made *Eps-317-1-E* more efficient in the use of soil moisture with increased transpiration efficiency (Asseng and Turner 2007; Meinke et al., 1997). Thus, introgression of the allele influencing early phenotype in this study into Australian barley cultivars may increase their adaptability to different environments

4.5 Conclusions

We used APSIM-Barley to conduct a GxExM analysis using four genotypes, ten sites, twenty years of climate data, four sowing dates, four levels of N fertiliser and three planting densities.

The allele (*Eps-317-1-L*) influencing higher yield in Northdown, Grenfell and Lexton was mainly due to the longer growing season at these sites. The early allele (*Eps-317-1-E*) had mean yield advantage over the late genotypes Franklin and *Eps-317-1-L* at Binnu, Denial Bay, Esperance, Pallamallawa, Yarrawonga and Grenfell most of these sites were relatively short growing seasons. The yield potential of the later genotypes at these sites was impaired due to greater biomass accumulation and longer life-cycles, resulting in longer depletion of plant available water and mineral nitrogen, and consequently resulting in an earlier onset of terminal water stress and cessation of grain-filling. With respect to sowing dates, yields were generally higher in May and June sowing compared with other sowing dates, although this also varied annually. This study has demonstrated that breeders need to match genotypic phenology to specific environment types, while breeders and farmers need to select alleles and genotypes in line with anticipated crop management practices. Thus, the alleles and new genotypes we have developed may be more preferable in regions of low rainfall and high temperatures, and in the case of *Eps-317-1-E*, be more suited to April, May and June sowing dates. *Eps-317-1-L* is more adapted to cooler conditions with higher rainfall due to slower growth rate and high tillering ability but also harvest index, which promulgates higher yields than Franklin. In future, modelling studies such as this could explore a range of traits other than phenology that may be manipulated in subsequent breeding experiments, such as light-saturated photosynthesis and tillering capacity.

Chapter 5 Quantitative trait loci for ear emergence in SYR01 x Gairdner double haploid DH) populations

Abstract

Heading date (HD) is an important agronomic trait that influences both plant adaptability to varying environment and grain yield. Using a double haploid population derived from a cross between a wild and a cultivated barley sown in three seasons differing in day length and temperature, we investigated quantitative trait loci (QTL) controlling heading date from different growing seasons in Launceston, Tasmania. Nine QTL were identified, which accounted for 43% of the phenotypic variation. Most of the QTL were co-located with previously reported genes for heading date. These QTL were located on chromosomes 2H (2), 3H (2), 5H (3), 6H (1) and 7H (1), respectively. The two QTL on 2H were located at similar positions to *Ppd-H1* and *Eam6/eps2S*, respectively. Two QTL on chromosome 5H were most likely an Eps gene reported earlier and *Vrn-H1/HvPHYC*. One of the QTL on 3H (*Qhd-sg-3H.2sm*), which was expressed in both winter and spring sowing, was located at a region where no genes were reported. More work is required to confirm the novelty of this QTL.

5.1 Introduction

Barley is an important cereal crop grown in Australia with environments with highly variable climates, including prevalent drought and heat stress (ABARES, 2017). Most growing regions of the crop in Australia are rain-fed. The most important adaptive trait is the time to ear emergence, which needs to occur after barley genotypes have already optimised production of biomass and yield potential (Boyd et al., 2003). The genotype selected for a specific environment is challenging (Cuesta-Marcos et al., 2008), and needs to account for sowing date, likelihood of frost if flowering too early, and terminal heat and/or water stress if

flowering too late. Genotypes with short basic vegetative phase (*BVP*) or early maturity are usually more desirable in environments prone to drought and heat stress challenges (Ibrahim et al., 2018; Shavrukov et al., 2017). A better understanding of factors and their interaction regulating ear emergence in barley could help in facilitating the selection of appropriate genotypes for adaptation. Being a complex trait, ear emergence is regulated by environment, physiological and genetic factors (Hill et al., 2016; Ibrahim et al., 2016) through which modifications can be can carried out to match the available resources.

Day length and temperature are two main environmental factors influencing heading date (Cockram et al., 2007). The genetic factors consist of three major groups of genes; photoperiod (*Ppd)*, vernalisation (*Vrn)* and *earliness per se* (*Eps* or *BVP)* (Boyd et al., 2003; Ibrahim et al., 2016). QTL analysis is very useful in the identification of genes and allele diversity regulating this trait for a specific adaptation (Casas Cendoya et al., 2008; Meszaros et al., 2008). Near isogenic line (NILs) and double haploid (DH) populations have been deployed to characterize and identify closely linked markers to the genes regulating ear emergence in barley (Boyd et al., 2003).

Photoperiod genes include *Pdd-H1* and *Pdd-H2* on chromosome 2H and 1H, respectively (Alqudah et al., 2014; Sameri et al., 2011). The *Pseudo-response regulator* (*HvPRR37*) called *Ppd-H1* is a major long day length response gene with a recessive allele (*ppd-H1*) being less sensitive to day length (Alqudah et al., 2014). The *FLOWERING LOCUS* (*FT*) gene, *Ppd-H2* (*HvFT3*) is a floral promoter in which the dominant allele induces heading under short days (displayed in Morex) while the recessive mutant *ppd-H2* (in Steptoe) has reduced sensitivity (Kikuchi et al., 2009). Response to prolonged low temperatures (vernalisation) in barley, is controlled by *Vrn-H1, Vrn-H2, Vrn-H3* and *Vrn-H4* located on chromosomes 5H, 4H, 7H and

5H, respectively (Hill et al., 2016). *Vrn-H1* (an *APETALA1* and *FRUITFULL-like* MADS-box transcription factor) on 5H is the key determinant of vernalisation (Cockram et al., 2007). Allelic variation in the locus is the cause of differences in levels of vernalisation requirement in barley (Hemming et al., 2009; Trevaskis et al., 2003). Similarly, *Vrn-H2* (a ZCCT zinc finger transcription factor) on 4H is a strong inhibitor of flowering under long-day in which the deletion in the dominant allele is responsible for the vernalisation requirement (Karsai et al., 2005). *VRN-H3* (*HvFTI*) on 7H is positively correlated with *Vrn-H1* in which mutation at the dominant allele leads to early flowering (Rollins et al., 2013). Epistatic interaction at three loci is the main cause of the growth habit where the vernalisation-responsive haplotype is *vrn-H1-Vrn-H2-vrn-H3* allelic combination (Karsai et al., 2005). Other loci that have effects on ear emergence are earliness per se (*Esp* or *BVP*) genes, which are located on every chromosome. These genes are fully expressed when photoperiod and vernalisation requirements are met (Laurie et al., 1995)

Wild barley genotypes show a diversity in various traits from cultivated barley. The aims of this study were to investigate the diversity in genes regulating heading date between wild (SYR01) and cultivated barley (Gairdner) and to identify new QTL/genes regulating ear emergence which could be delivered to breeders.

5.2 Material and methods

5.2.1 Plant materials

A total of 173 double haploid (DH) lines developed from a cross between a Syrian wild barley (*SYR01*) and an Australian cultivar (*Gairdner*) were used for this experiment. The wild Syrian genotype is a winter type while Gairdner is a medium maturing barley with prostate growth habit at early stage and medium sensitivity to day length (Smith et al., 2005).

5.2.2 Phenotyping

Field experimentation was conducted in 2017 at Mt Pleasant Laboratories, Tasmanian Institute of Agriculture, Tasmania, Australia (41.4667°S, 147.1500°E). All DH lines and the two parents were sown in a randomised complete block design using three sowing dates, winter (15 May 2017), spring (15 October 2017) and summer (15 November 2017) to evaluate the heading date under contrasting environment and rain fed conditions. Supplementary irrigation using sprinkler was provided in spring and summer when needed.

Each genotype was planted in one row of 100 cm long with a row spacing of 25 cm. Fertiliser (150 kg/ha of 5:10:10:5 N:P:K:S) was applied at sowing while 100 kg urea/ha was applied at GS45.

5.2.3 Map construction and QTL analyses

DH lines and the two parental varieties were genotyped with DArTSeq (http://www.diversityarrays.com/dart-application-dartseq). Due to the large number of markers (~30,000 SNP and DArTSeq markers), those with the same positions or with greater distortion and missing data were removed from the map construction. 3363 markers were used to construct the map using JoinMap 4.0 (Van Ooijen 2006). The marker positions were then aligned with the consensus map (Mascher et al., 2017).

QTL mapping was conducted using MapQTL 6.0 software (Van Ooijen, 2006). Interval mapping (IM) was first conducted. The closest marker at each putative QTL- identified using interval mapping was selected as a cofactor and the selected markers were used as genetic background controls in the approximate multiple QTL model (MQM). A logarithm of the odds (LOD) threshold values applied to declare the presence of a QTL were estimated by

performing the genome wide permutation tests using at least 1000 permutations of the original data set for each trait, resulting in a 95% LOD threshold of around 3.0. The percentage of variance explained by each QTL (R^2) was obtained by using restricted MQM mapping. The sequence of peak DArT and SNP markers linked with heading date were used for the blasting on the IPK search website: (http://webblast.ipk-gatersleben.de/barley/) to identify the homologous or corresponding position of the peak marker in the morex_contig. MapChart 2.2 was then employed for the linkage map according to the method described by Voorrips (2002).

5.3 Results

5.3.1 Phenotypic variation of the SYR01 x Gairdner DH population for heading date

There was a significant difference between the parents for heading date and among the DH populations. SYR01 was earlier than Gairdner in winter sowing but later than Gairdner in both spring and summer sowing (Fig. 5.1). Continuous variation in heading date was observed among the DH lines. We observed a wide range of heading date for the three sowing dates (Fig. 5.1).

Heading date of the DH lines ranged from 140 to 174 DAS (days after sowing) in winter sowing, 43 to 100 DAS in spring sowing and 45 to 98 in summer sowing trials (Fig. 5.1) with summer trial showing the shortest time from sowing to heading (Fig. 5.2), although there were no substantial differences in heading date between lines sown in spring and summer. Similar results were found after converting to GDDs to heading (Fig. 5.3). Similarly, the principal component analysis (PAC), indicated that component accounted for around 57% of the total variance while component 1 explained 23% of the variation (Appendix Fig 8.1).

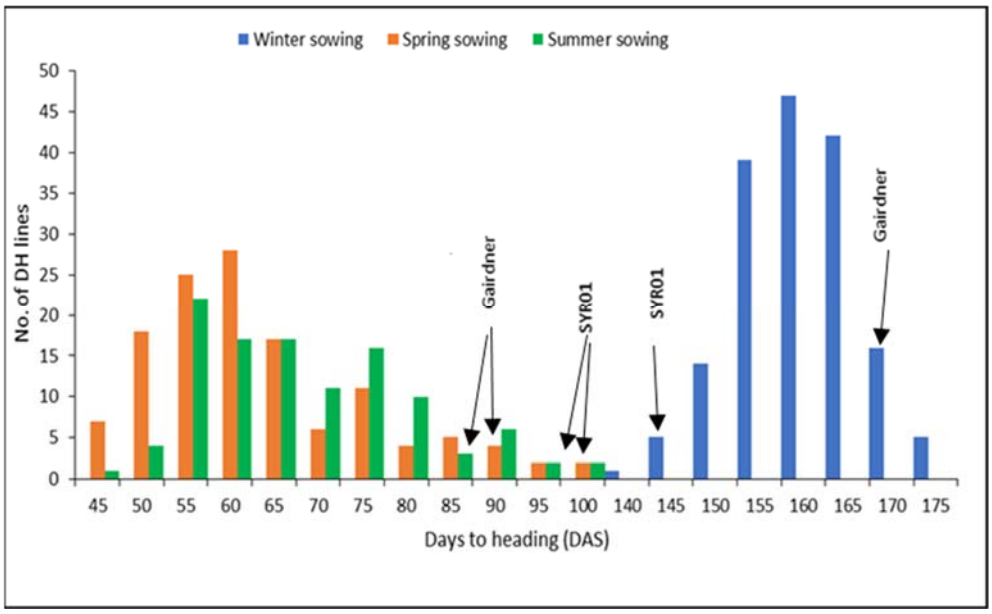

Figure 5.1 Frequency distribution of heading date of SYR01 x Gairdner DH populations for winter, spring and summer sowing. The vertical arrows show the parents of the DH lines.

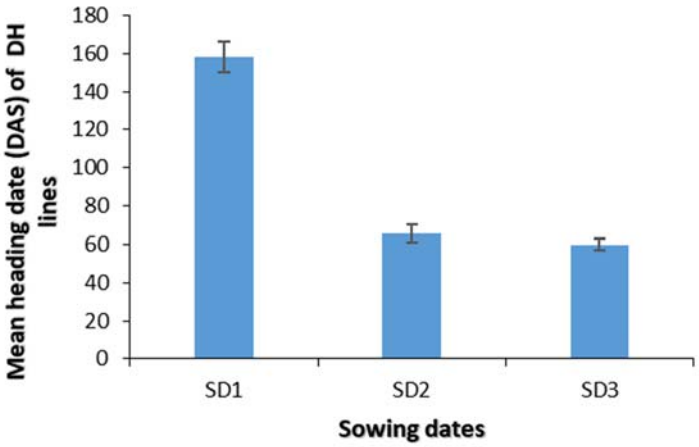

Figure 5.2 Frequency distribution of SYR01 x Gairdner DH populations for heading date for the three sowing dates.

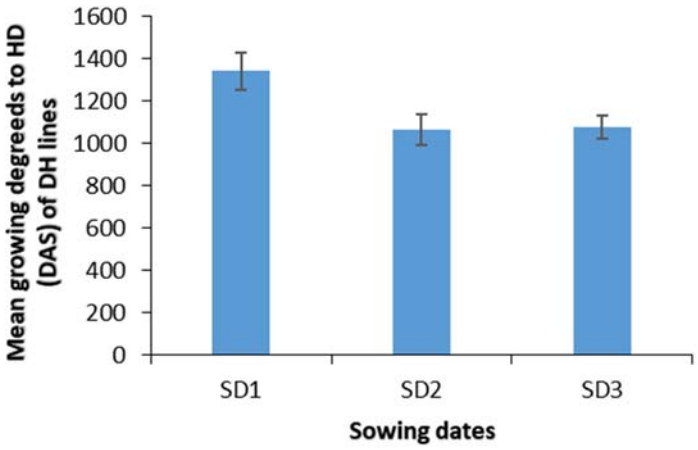

Figure 5.3 Frequency distribution of SYR01 x Gairdner DH populations for growing-degree-days for the three sowing dates.

5.3.2 Quantitative trait loci for heading date

Ten QTL for heading date were identified with phenotypic variance from 6% to 47% across the three sowing dates (Table 5.1). As shown in Figure 5.4, two QTL were identified on chromosome *2H, Qhd-sg-2H.1w* and *Qhd-sg-2H.2s*. The peak marker for *Qhd-sg-2H.1w* is 11288299D at 43.7 cM (Tables 5.1 and 5.2). This QTL was only identified from winter sowing trial. The second QTL, *Qhd-sg-2H.2s*, was identified in spring sowing and is located at 18.9 cM with 3986258D2 being the closest marker. On 3H, we found two QTL. The first one, *Qhd-sg-3H.1w*, was identified in both winter and summer sowing trials with 3254867S3 being the closest marker at 108.4 cM (Table 5.1). The other QTL, *Qhd-sg-3H.2sm* is located close to 4596019D3 at 133.0 cM and was identified only in the summer sowing. One QTL, *Qhd-sg-4H.1s,* was found on 4H with 3664225D4 being the peak marker, which is located at a similar position to *Vrn-H2*. Three QTL, *Qhd-sg-5H.1s*, *Qhd-sg-5H.2s* and *Qhd-sg-5H.1sm,* were identified on 5H. These QTL were located at 44.14, 154.8 and 112.71 cM, respectively. We also identify one QTL on 6H and 7H with 3269813D6 and 5240745D7 as the peak markers,

respectively. The sequence of the most significant marker is displayed on (Table 5.2). The Blast analysis in the IPK site showed the variant morex_contig with the corresponding positions of both the DArT and SNP markers detected this study (Table 5.1).

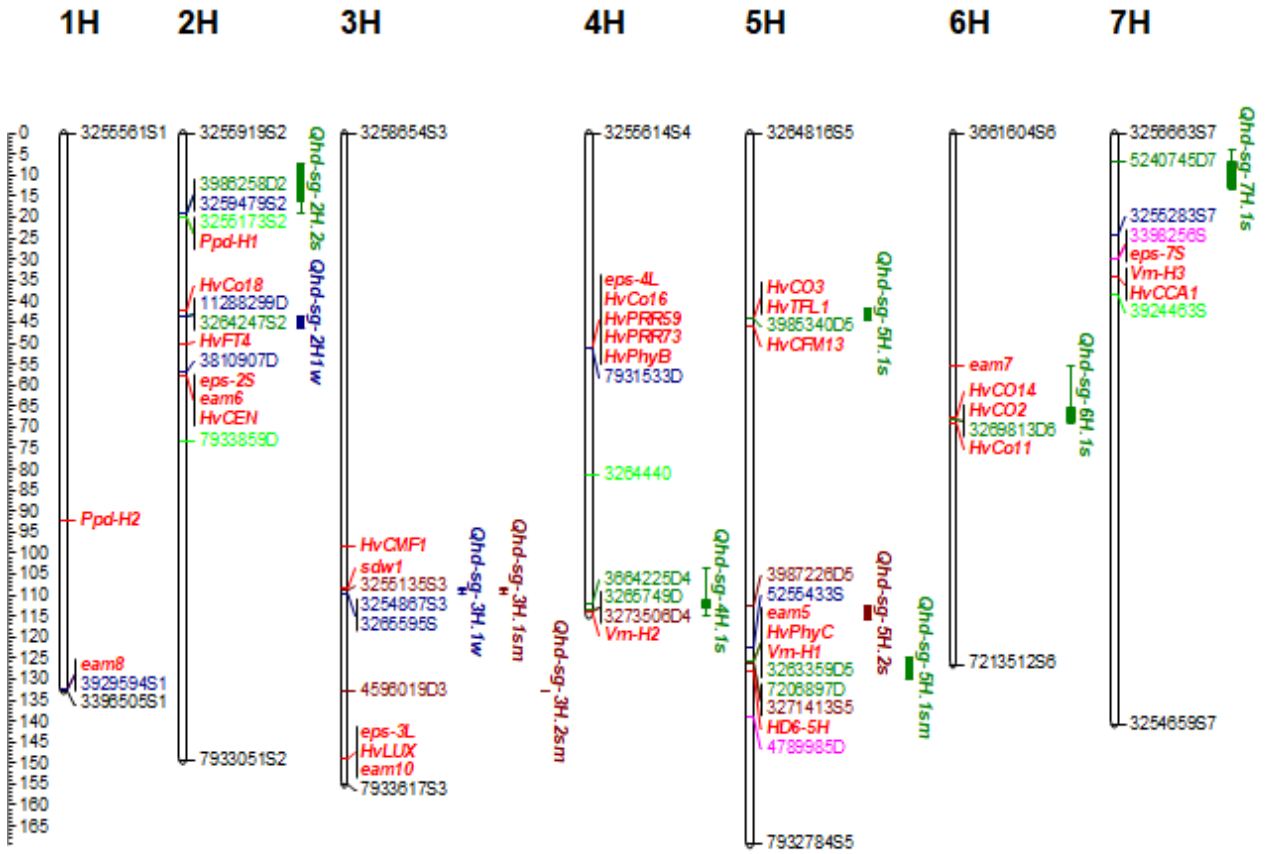

Figure 5.4 Linkage map of anchored SNP and DArT markers with their relative positions for heading date of barley SYR01 x Gairdner DH populations. Colours depict the season: blue winter (w), green spring (s) and pink summer (sm) while the red colour means the position of the reported genes.

Table 5.1 The QTL, Peak SNP and DArT markers with their positions. The morex_contig, position and sequence used in the QTL analysis for ear emergence and other agronomic traits in the SYR01 x Gairdner DH populations. R2 means percentage genetic variance explained by the nearest marker, while LOD mean the logarithm of the odds (LOD) threshold values.

QTL Name	Linkage group	Peak markers	Peak Position (cM)	LOD	R^2	Variant (Morex_contig)	Consensus Position (cM)
Qhd-sg-2H.1w	2H	11288299D	46.53	11.66	22.1	morex_contig_60446	43.7
Qhd-sg-2H.2s	2H	3986258D2	26.46	17.36	47.1	morex_contig_48296	18.9
Qhd-sg-3H.1w	3H	3254867S3	70.78	14.27	26.1	morex_contig_61213	108.4
Qph-sg-3H.1sm	3H	3255135S3	72.0	18.22	40.0	Morex_contig_1577872	108.57
Qhd-sg-3H.2sm	3H	4596019D3	88.03	3.63	7.0	morex_contig_51848	133
Qhd-sg-4H.1s	4H	3664225D4	134.97	3.14	6.5	morex_contig_1579833	112
Qhd-sg-5H.1s	5H	3985340D5	23.85	3	5.9	morex_contig_2552471	44.17
Qhd-sg-5H.2s	5H	3263359D5	214.18	6.29	13.9	morex_contig_136101	125.8
Qhd-sg--5H.1sm	5H	3987226D5	86.52	4.31	11.1	morex_contig_159035	112.71
Qhd-sg-6H.1s	6H	3269813D6	85.337	3.03	10.3	morex_contig_8211	68.2
Qhd-sg-7H.1s	7H	5240745D7	3.15	3.89	7.4	morex_contig_2546573	6.76

Table 5.2 The QTL, the morex_contig, and sequence used in blasting at IPK website of the QTL analysis for ear emergence and other agronomic traits in the SYR01 x Gairdner DH populations

QTL Name	Variant (Morex_contig)	Allele sequence
Qhd-sg-2H.1w	morex_contig_60446	TGCAGCTAACCAGTGTGATTCGGATGCGCGACACTGGAGACTGACAAAAAATTCGTTTTGCTTTCTAGT
Qhd-sg-2H.2s	morex_contig_48296	TGCAGCCGGCGAGGTGGCTAGTTATATGGCTCACGACGGGCGGTCTCCGTGCACGGGATGGGGATCCGA
Qhd-sg-3H.1w	morex_contig_61213	TGCAGGATTGAATTGCTGCCCACATGGTCATCCAGAATACCTACACCGAGATCGGAAGAGCGGTTCAGC
Qhd-sg-3H.2sm	morex_contig_51848	TGCAGACCATGTTGACCTCGCGAATACATAGCTAGGGTACACAAGGAAAACAAAGGGGAGAATGGTGAT
Qph-sg-3H.1sm	Morex_contig_1577872	TGCAGCATTCCCATTAACGAAGGCATCTTAATTACTCTGCTGTTACACATGGCCTAAACTAAATGAGGC
Qhd-sg-4H.1s	morex_contig_1579833	TGCAGTAGCGACTCTGACCGTCGTCTCTTAACATTATACAAGCGGCAGTAGAACGAGTACCCTTTTCCG
Qhd-sg-5H.1s	morex_contig_2552471	TGCAGCACTAGCGGCAACCTATGTTCATCATCACGGCGGAGCAACAACGCAACATGTGTGCTGCTTCAA
Qhd-sg-5H.2s	morex_contig_136101	TGCAGACTGCATACAAGGAGCATGACAGACTGAACGGAGATCACACCAATCACCCGATTCACCGAGATC
Qhd-sg-5H.1sm	morex_contig_159035	TGCAGCCTGAAGATACGATGGATCGTCGAACGAAGCCGGCCAGCCATCATGGCCGCCATGGCTCCTCCA
Qhd-sg-6H.1s	morex_contig_8211	TGCAGCAAAAAATCCCTATAAAAAACACCCCATTACAGATGGGGCGCCGAGATCGGAAGAGCGGTTCAG
Qhd-sg-7H.1s	morex_contig_2546573	TGCAGCACGACGACATACCCAAGCTGAAGTACCTGAAGATGGTGGTGAAGGAGACGCTGCGGCTGCATC

5.4 Discussion

Heading date is an important agronomic trait for barley and can help maximise yield potential if timing is appropriate to environment. The genetic basis of heading date in barley has been investigated in many studies, with numerous genes being detected over the whole barley genome. For example, photoperiod (Alqudah et al 2014), vernalisation (Cockram et al 2007) and *earliness per se* genes (Lewis 2008). The wide chromosomal distribution of heading date related QTL indicates the complex regulatory networks and great number of genes controlling this trait. Several pathways promote heading and flowering of barley, including vernalisation, photoperiod and autonomous and gibberellin pathways (Boss et al., 2004). Previous studies have identified a large number of QTL/genes for heading date (Backes et al., 1995; Cuesta-Marcos et al., 2008; Kjaer et al., 1995). However, most of these efforts focused on the QTL expressed under a single environment. In this study, the approach of different sowing times enabled the identification of QTL potentially operating in different regulatory pathways and then using principal component analysis (PCA) to understanding the relationship between the growing conditions and heading date. We have also used a cross between a wild barley accession and a cultivated barley, which resulted in a much greater number of QTL. Based on this different growing condition, component 1 of the principal component analysis (PCA) explained around 57% of the total variance for heading date majorly from winter sowing (Figure S8.1). Although heading of the genotypes for all the three growing seasons area positively correlated, heading in spring and summer sowing are more closely related than with winter sowing, indicating similarity in the genes regulating heading date in the two growing seasons (spring and summer).

We identified nine QTL in the SYR01 x Gardiner DH population from different sowing dates. Most previously identified major genes were found in this DH population.

On chromosome 2H, the first QTL (*Qhd-sg-2H.1w*) is located at around 43.7 cM of the short arm linked with 11288299D DArT marker. This region coincides with the position of *Eam6/eps2s* (Alqudah et al., 2014). Similarly, a QTL for heading date with *eam6* as candidate was detected around this region in autumn sown conditions (Comadran et al 2011)**.** *HvC018* (Nice et al., 2017) and *Eps2S* (Sameri et al., 2011). Although this gene is thermo-sensitive (Appendino et al., 2003), it is fully expressed after the requirements for photoperiod and vernalisation are met (Alqudah et al., 2017; Zikhali et al., 2015). The second QTL (*Qhd-sg-2H.2s*) is on the short arm of chromosome 2H, at a similar position to *Ppd-H1* identified under long day (Alqudah et al., 2014; Laurie et al., 1994; Laurie et al., 1995; Luna Villafaña, 1995; Wang et al., 2014), and accounted for 22% of the phenotypic variance. This QTL was detected only in the spring sowing trial indicating that this is a photoperiod sensitive gene. However, allelic variation at *Ppd-H1* locus was associated with a minimum difference of 2 days in heading date in the winter (Digel et al 2016).

On chromosome 3H, we identified two QTL at the long arm region. One of the QTL (*Qhd-sg-3H.1w*) was identified in both winter and spring sowing. This QTL is located at 108.4 cM linked with 3254867S3 SNP marker in winter and 3255135S3 marker in spring (Table 5.1) and accounted for about 26% of the phenotypic variance. Similarly, Alqudah et al. (2014) identified a HD QTL linked with (*i_SCRI_RS_14857*) marker around 105.0 cM in world barley collections. A QTL influencing heading date and plant height, which has an effect on prostrate growth habit as observed in this study (especially displayed by Gairdner), has been reported on 3H (Powell et al., 1985; Ponce-Molina et al., 2012). Also, in line with this study, Cuesta-

Marcos et al. (2008) obtained a QTL close this location (116.0 cM), by *E35M47_k* marker with significant effect on effect on HD in a spring x winter DH lines of Mediterranean origin. Furthermore, Yin et al., (1999) identified a QTL with considerate effects on pre-flowering date. The second QTL (*Qhd-sg-3H.2sm*), which was also expressed in both winter and spring sowing, was at 133.0 cM with a peak marker of 4596019D3, where no genes were reported in this region. However, some QTL for HD were reported in this region (Wang et al., 2010). Bauer et al. (2009) also described a QTL for HD with a closest marker of *GBM1043* at 130.0 cM. Similarly, Yin et al. (1999) reported a HD QTL, which was 7.0 cM away from the position observed in this study. In addition, Von-Korff et al. (2006) observed a similar ear emergence QTL around this region by *HV13GEIII* marker at 155 cM, which coincide with a position of HD QTL in a consensus map of Varshney et al. (2007). The QTL identified (*Qhd-sg-4H.1s*) on 4H at 112.0 cM was closely linked with 3664225D4 where a QTL was mapped at a similar position from the Galleon x Haruna Nijo DH population (Ren et al., 2010) and the Beka x Mogador DH (Cuesta-Marcos et al., 2008). This is more likely the vernalisation response gene, *Vrn-H2*, which was first, characterised and mapped to 4HL (Takahashi et al., 1971) and later *HvZCCT* was named as the candidate gene (von Zitzewitz et al., 2005).

Three QTL (*Qhd-sg-5H.1s*, *Qhd-sg-5H.2s* and *Qhd-sg-5H.1sm*) were identified on chromosome 5H. *Qhd-sg-5HS.1s* is located in a similar position to an *Eps* gene (Laurie et al., 1995), which has little available information. Both *Qhd-sg-5HL.2s* and *Qhd-sg-5HL.1sm* are in the region where *Vrn-H1* and *HvPHYC* (Nishida et al., 2013) are located. QTL have also been reported in this region from different populations (Boudiar et al. 2016; Ren et al., 2010). Functions and epistatic interaction of the alleles of this gene and other loci has been well documented (Szűcs et al 2007)**.** It has been reported that only the dominant *Vrn-A1* alleles in wheat has the

spring growth habit which is due to large deletions in its promoter region compared with other alleles (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*) of the locus, however, *Vrn-H1* in barley alleles show no polymorphisms in the promoter region compared to their respective recessive alleles (Fu et al 2005). Our earlier study also identified a new regulator for heading date (Chapter 3), which is located at the same position.

The observed QTL (*Qhd-sg-6H.1s*) on chromosome 6H had a minor effect, which accounted for only 10% of the phenotypic variance. The position of this QTL is coincident with previously reported *Eps* gene (*eps6L.1*) (Laurie et al. (1995). A gene with a similar effect on this chromosome was also reported by Cuesta-Marcos et al. (2008).

The QTL on chromosome 7H (*Qhd-sg-7H.1s*) was identified on the short arm from spring sowing. This QTL is similar to the previous reported QTL at the vicinity of *eps.7S* (Laurie et al., 1995; Yan et al., 2006) Another major QTL which requires vernalisation is also reported at this location, although initially mapped to 1H (Yan et al., 2006) and later reported on 7H (Faure et al., 2007). However, because of the minor effect of this QTL (7%), we envisage that *Eps7S* (found in spring sowing) might be the candidate of this QTL (Cuesta-Marcos et al., 2008; Zikhali et al., 2015).

In conclusion, the wild barley accession and cultivated barley showed a wide diversity in genes regulating plant development with a large number of QTL being identified from a cross between a wild barley and a cultivated barley. These QTL covered most of the reported genes for heading date, including two major vernalisation genes. There was one potentially new QTL for heading date, located on 3H (*Qhd-sg-3H.2sm)*, which needs to be confirmed for its novelty and usefulness in breeding programs.

Chapter 6 General discussion and conclusion

Barley is widely distributed and adapted cereal crop in the world and is best known for its use as feed and malting (Ullrich, 2010). Phenological traits such as heading date and GDD are important in adapting barley to diverse environmental conditions. The significance of these traits for the adaptation is due to diversity of genes (polygenic) that are involved in their regulation (Cockram et al., 2007; Li, 2018). This study focused on maturity regulators in barley because of their importance in mitigating the threats from climate variability including drought and heats stresses that cause considerable loss in grain yield and quality. Identification and characterisation of these genes will facilitate breeding efforts for increased yield and quality.

6.1 Identification of a QTL regulating early maturity from the TX9425 x Franklin population

Variability in days to heading and GDDs have been reported in barley genotypes (Cockram et al., 2007; Dai et al., 2012). The first early maturity regulator on chromosome 5H was reported by Wexelsen (1934) and subsequently confirmed by Laurie et al. (1995). Subsequent reports also indicated presence of the following maturity genes *Vrn-H1* (Fu et al., 2005), Eps/HvPHYC (Alqudah et al., 2014; Nishida et al., 2013). In this experiment, a QTL regulating ear emergence was identified on chromosome 5H from the cross between TX9425 (a Chinese feed barley) and Franklin (an Australian malting barley). The QTL consisted of an early heading regulator, which is inherited from the early maturing parent TX9425. Comparing the position of the QTL in this study with the physical maps in Mayer et al. (2012), the locus coincides with location of *Vrn-H1*, a vernalisation gene (Cuesta-Marcos et al. (2008) and two other flowering regulators, HvPHYC and HvCK2α-5H (Pankin et al., 2014; Nishida et al., 2013).

Detailed analysis of the position of the *Vrn-H1* gene by Yan et al. (2003) did not show the presence of any other gene around 324-kb between the *Vrn-H1* flanking genes *Cysteine* and *Cytochrome B5*, with *Vrn-H1* having strong linkage (0.03cM) to MADS-box genes *AP1* and *AGLG1.* The dominant allele of *Vrn-H1* does not respond to vernalisation (Fu et al. 2005), which is as result of large deletions at the first intron overlapping in a 4-kb region of the allele. The recessive allele, *vrn-H1,* which has no deletion, has strong vernalisation response (Cockram et al., 2007). The allele of the gene in this study did not require vernalisation before induction of flowering indicating that gene is different *vrn-H1,* which needs plants to expose to prolonged cold temperatures before switching to reproductive phase (Loukoianov et al., 2005).

Another gene at similar position is the *Casein kinase 2* (*HvCK2α-5H*) which is serine/threonine phosphotransferase (Kato et al., 2008). *CK2* has been demonstrated to affect clock protein in *Arabidopsis* (Daniel et al., 2004) while, in rice, the *Hd6* QTL regulating HD encodes the alpha (-*α*-) subunit of the CK2 (Takahashi et al., 2001). However, in barley, *HvCK2α-5H* is a light receptor gene expressed in long days and is flanked by WG644 and ABG712 (Kato et al., 2008; Nishida et al., 2013) and therefore this gene is not the candidate for the QTL we identified.

Another possible candidate is the *HvPHYC*, which is also a photoreceptor gene closely linked with *Vrn-H1* (Kato et al., 2008). Similar to the observed regulator in this study, both alleles are functional with *HvPhyC-e* and *HvPhyC-l* causing early and late flowering respectively (Nishida et al., 2013). However, the up-regulation of *HvPhyC-e* through *FLOWERING LOCUS T1* and *CONSTANS1* occurred only in long daylength (Nishida et al., 2013) while the QTL reported in this study expressed in both short and long daylength. In addition, for *HvPhyC-e* to accelerate

flowering under short day, it has to interact with *Eam1* (*Ppd-H1*) (Pankin et al., 2014) indicating that the observed gene is not *HvPHYC*.

6.2 A wild barley accession showed the difference in most of the known genes for heading dates from the cultivated barley Gairdner

Plant progression from vegetative to reproductive (heading date) is mainly regulated by three kinds of genes, photoperiod (*Ppd*), vernalisation (*Vrn*) and *earliness per se* (*Eps*) (Laurie et al., 1995; C. Li, 2018) (Ibrahim et al., 2018b). A total of nine QTL were identified from a DH population originated from the cross of SYR01 x Gardiner including the major flowering genes (*Pdd, Vrn and Eps*). In this study, two QTL were identified on the short arm of chromosome 2H. The first QTL was identified in winter (*Qhd-sg2H.1w*), which is located at around 43.7 cM of the short arm linked with 11288299D DArT marker. This region coincides with the position of *Eam6/eps2s* (Alqudah et al., 2014) and *HvC018* (Nice et al., 2017). The second QTL (*Qhd-sg-2H.2s*) was found around the region of 18.9 cM, similar to the position of *Ppd-H1* identified under long day conditions (Alqudah et al., 2014; Laurie et al., 1994; Laurie et al., 1995; Luna Villafaña, 1995; Wang et al., 2014). We found two QTL for heading date at the long arm region of chromosome 3H. The first QTL (*Qhd-sg-3H.1w*) identified in both winter and spring sowing precisely at 108.4 cM linked with 3254867S3 SNP marker in winter and 3255135S3 marker. This region is similar to the position of a HD QTL around at 105 cM using diverse panels of spring barley (Alqudah et al., 2014). More evidence of a QTL for heading date found at a similar a position described in this study have been described reported (Powell et al., 1985; Ponce-Molina et al., 2012; Cuesta-Marcos et al., 2008). The second QTL (*Qhd-sg-3H.2sm*), which was also expressed in winter and spring sowing, was at the region of 133.0 cM with 4596019D3 as the peak marker. A major HD QTL has not been reported in this position,

however, many reports have shown that a HD QTL exist around this region (Bauer et al., 2009; von Korff et al., 2006; Yin et al .1999; Varshney et al., 2007). We identified one QTL (*Qhd-sg-4H.1s*) on chromosome 4H at 134.98 cM closely linked with 3664225D4 where a QTL was mapped at a similar position from the Galleon x Haruna Nijo DH population (Ren et al., 2010) and the Beka x Mogador DH (Cuesta-Marcos et al., 2008). This is more likely the vernalisation response gene, *Vrn-H2*, which was first characterised and mapped to 4HL (Takahashi et al., 1971). Three QTL (*Qhd-sg-5HS.1s*, *Qhd-sg-5HL.2s* and *Qhd-sg-5HL.3s*) were identified on chromosome 5H. *Qhd-sg-5HS.1s* is at a similar position to an *Eps* gene (Laurie et al., 1995). *Qhd-sg-5HL.2s,* linked to DArT marker 3263359D5, and *Qhd-sg-5HL.3s,* linked with 3987226D5, were found on the long arm at a position 125.8 and 112.7 cM, respectively, where *Vrn-H1* and *HvPHYC* (Nishida et al., 2013) are located. A QTL with minor effects was observed on 6H, which explained about 10% of the phenotypic variance. The QTL coincide with eps6L.1 reported by Laurie et al. (1995). One QTL (*Qhd-sg-7Hs*) was identified on the short arm of chromosome 7H, which is likely to be an *Eps* gene (Laurie et al. (995). This QTL also has small effect 7% like other Eps genes (Zikhali et al., 2015)

6.3 Modelling the effects of phenological traits to suit diverse environmental and management conditions

To determine the performance of the four barley genotypes used in this study to different environments in Australia, we used APSIM-Barley to characterise the phenological and yield performance of the genotypes across a range of environmental conditions and management practices. The simulations confirm the significance of matching crop flowering date to a specific environment and management practices for the new breeding lines. For instance, the late heading genotype *Eps-317-1-L* had higher GxE interaction with higher grain yield followed by Franklin in some of the environments. The performance of the *Eps-317-1-L* was due to

adequate plant available water content and optimum temperature in these environments, which resulted in high tiller numbers and greater biomass production compared with other genotypes. Franklin, which is also a late genotype typically, had greater tiller proliferation most of which were unproductive due to higher competition for the available resources. Apart from impairment of photosynthesis due to shading effects by the older tillers, the prolific tillers production in Franklin may have resulted in faster depletion of soil water and nitrogen and thus earlier onset of N stress, affecting leaf expansion, canopy photosynthesis and the extent of grain filling, particularly in dry years such as 2016. This observation agrees with previous studies such as Meinke et al. (1997) in a wheat experiments where low soil nitrogen during early phenological stages inhibits leaf expansion, affecting the photosynthetic rates and therefore the amount of photosynthate available for grain filling. Our results confirm these findings for all genotypes, and TX9425 in particular. During heading and anthesis, about 10-60% yield loss can be attributed to nitrogen stress in crops when soil water is adequate (Sadras et al., 2016). This indicates that the physiological outcome of the stress depends on its severity and the growth stage of the genotype, with the flowering window being a crucial period for farmers to minimise stresses to maximise yield potential (Damisch et al., 1991). The simulations conducted in this thesis indeed indicated that, in some seasons, N stress acting on leaf expansion, phenology and photosynthesis can impede optimal development of crop yield. The simulations conducted in this thesis were conducted with realistic farmer management for fertilisers. Future experiments should examine how results differ with unlimited application of N throughout the crop life cycle.

6.4 Response of new and existing barley genotypes to different environments

Environment (sites and years) had significant effects on floral initiation, heading date and grain yield. GxE interaction effects can mask the breeding and genetic value new genotypes in a multi-environmental experiment (Zorić et al., 2017). The results in this study emphasis the need to consider the importance of environment in selecting genotypes for target populations of environments (Chapman et al., 2000; Chapman et al., 2002). For instance, cultivars required ~135 days to head in Northdown compared with ~69 days at Capella. Greater rate of degree-day accumulation caused the earlier phenological development in Capella, Binnu and Pallamallawa. Previous studies have shown that GDD is an important driver of plant organ development and growth from germination to maturity (Boote et al., 2013). Crop performance can also be affected by soil type (Sassenrath et al., 2015), and this can be exacerbated by other soil-related stress factors depending on cultivar sensitivity (Slafer et al., 1995). The combination of low rainfall and relatively high temperature at these sites hastened water loss through evapotranspiration. Other studies have also suggested that high temperatures at anthesis have negative effects on yield due to increased pollen sterility, reduced ovule viability and spikelet abortion (Hasanuzzaman et al., 2013).

6.5 Response of new and existing barley genotypes to management

The use of appropriate management options for better-adapted cultivars in different production environments is essential for increasing and maintaining the reliable crop productivity (Wang et al., 2001). The variation in heading date and yield in this simulation underscores the strong effects of the management. Thus, to maximise yield, optimising

sowing times for each genotype is paramount so that ear emergence can occur during lowest long-term frequencies of frost and drought. We show that sowing in May and June generally had similar effects on ear emergence and resulted in higher grain yield. This is as result of optimum growing conditions, which includes good ambient temperature and adequate moisture for growth and development over the long-term. A recent report indicated that early planting in NE Australia (May to June) may provide better grain yields and quality with lower protein levels making more attractive to malting industries (QDAF, 2018).

Sowing early in some years may also be detrimental to productivity, however. In some simulations in which sowing was performed earlier (e.g. March), the late genotypes *Eps-317-1-L* and *Franklin* experienced more soil water and nitrogen stress in April compared with other genotypes (data not shown), which hastened flowering and truncated later potential biomass accumulation. In such cases, longer varieties would be better sown in late April (mid-autumn).

Another vital management option is nitrogen fertilisation, which is essential for growth and development and ultimately results in optimal grain yields and quality (Lei et al., 2018; QDAF, 2018). However, our study showed little effect of N fertilisation on phenology, in agreement with results reported by McGuire et al. (1979). Increased nitrogen level under water non-limiting conditions increases yield, which is attributed to higher biomass accumulation, including higher number of tillers. This is particularly important because increased nitrogen levels have significant effects on leaf expansion, and consequently leaf area index. Higher leaf area index (up to a point) generally leads to an increase in light interception, which in turn increases plant growth, biomass and grain yield (Meinke et al., 1997).

6.6 Breeding for crop adaptation

In the past, breeding efforts for adaptation involved intensive selection of best performing genotypes under non-stressed target environment with the anticipation that the selected genotypes would be adapted and be superior in any production environment (Muñoz et al., 1998).

Recent paradigm shift occasioned by the challenges of the GxE interaction involves attempts to genetically improve yield through matching genotype and management to specific environments (specific adaptation). In this simulation, the genotypes responded differently in all the traits across sites. For instance, when looking at the comparative yield advantage, TX9425 was the earliest to mature but had the lowest yield across environments except Denial Bay and Pallamallawa, while *Eps-317-1-E* was generally more moderately to broadly adapted to environments and management practices examined here. Genotypes with faster growth rate have the ability to accumulate assimilate rapidly before the onset of the drought (Al-Ajlouni et al., 2016). Here, we show that the success of *Eps-317-1-E* could be due to its later ear emergence, but similar maturity date compared with TX9425, a faster growth rate and relative smaller leaf area index that made *Eps-317-1-E* more efficient in the use of soil moisture (Asseng et al., 2007; Meinke et al., 1997). Furthermore, transpiration efficiency (*TE*) is often seen as an opportunity for crop genetic improvement in drought prone environment to achieve higher yield (Turner et al., 2001). Apart from *TE*, *WUE* is universally accepted as a target physiological trait of interest for genotypes in drought prone environments (Muñoz et al., 1998). The genomic resources within the *Eps-317-1-E* genotype may have contributed to its broad adaptation and thus could help in the expansion of its cultivation from the traditional site of adaptation to other sites. The late genotype, of the NILs, *Eps-317-1-L,* had better yield

in long-season environments, Northdown and Lexton, with high rainfalls and cooler terminal temperatures. This performance could be attributed to the adequate plant available water in those sites and years, which might have caused increased biomass production. The genotype also had enough time to partition assimilates for grain development especially during the grain fill period. Results from this study could help in the understanding and identification of marker ideotype, which be a paradigm shift in barley breeding.

6.7 Future research

Determination of the natural diversity of maturity genes, and their allelic variations could provide an opportunity to better fine tune heading dates in different production environments, which can be a critical factor in adaptation and consequently yield in barley.

The identified QTL on chromosome 5H in this study causes variation in heading date in both long and short day conditions. This region of the QTL needs to be further fine-mapped for possible cloning to identify the closely linked marker, which could be used in breeding programs. More studies are also required to help understand intra- and inter-locus interactions of the gene and to determine how it responds to different environment in its interactive stage (polygenic condition). Apart from yield, quality traits are also important in determining the commercial values of barley, thus more research will be needed to investigate the influence of this gene and other maturity genes on both physical and biochemical traits. It will also be important to extrapolate performance of new genotypes to diverse conditions and dissect GxExM using crop modelling.

A total of nine QTL for heading date were identified from a population between a wild barley and cultivated barley, with most of them co-located with previously reported genes/QTL. Further studies are needed to confirm if these QTL are the same as previously reported,

preferably through NILs. The novelty of the new QTL for heading date on chromosome 3H also needs to be validated.

Since QTL or genes decide the phenotype in a given environment (Alberch, 1991), breeders find it challenging to extrapolate the QTL information from a group of environment and management to a new environment (Stratton, 1998). Further modelling is required to partition and explain the contribution individual components of the genotype and environment on the observed phenotype and the consequences of this for quality and yield. In addition, further research should involve combining QTL-mapping and simulation to dissect the complexity surrounding specific allelic responses and response of allelic combinations and non-allelic interactions with environment and management (Li, 2018; Yin et al., 2005). Simulation of the responses of the QTL and alleles regulating high value physiological traits such as *WUE* and *TE* with environment and management is also required.

Chapter 7 References

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Chapter 8 **Appendix 1**

Table 8.1 Genetic Coefficient fitted for APSIM-Barley Calibration including the model parameters and their values for the defaults (original values) and each of the genotypes

Cultivar parameters	Description	Units	Cultivar				
			Default	TX9425	Franklin	Eps-317-1-E	Eps-317-1-L
tt_emergence units="oCd">45.0</tt_emergence	Thermal time needed from sowing to emergence	oCd	85	85	300	45	95
tt_end_of_juvenile units	Thermal time needed from sowing to end of juvenile	oCd	350	230	150	250	270
tt_floral_initiation	Thermal time from floral initiation to flowering	oCd	560	400	530	550	450
tt_flowering units	Thermal time needed in anthesis phase	oCd	35	150	250	65	200
tt_start_grain_fill	Thermal time from start of grain filling to maturity	oCd	350	180	545	320	380
fr_lf_sen_rate	Fraction of total leaf number senescing per main stem		0.035	0.05	0.035	0.05	0.05
grains_per_gram_stem	Grain number per stem weight at the start of grain filling	g	25	35	49	45	42

potential_grain_growth_rate	Potential daily grain filling rate	g grain^{-1}day^{-1}	0.001	0.003	0.90	0.05	0.038
max_grain_size	Maximum grain size	g	0.065	0.058	0.054	0.058	0.095
y_extinct_coef		g/MJ	0.4, 0.4, 0.4	0.23, 0.5, 0.0	0.24, 0.4, 0.6	0.4, 0.32, 0.32	0.2,0.4,0.4

The radiation use efficiency (*y_rue units*) is (0, 1.3, 1.3, 1.3, 1.3, 1.3, 1.3, 1.3, 1.3, 0 and 0 for *TX9425),* (0, 0, 1.2, 1.2, 1.2, 1.2, 1.2, 1.2, 1.2, 0 and 0 for *Franklin*), (0, 1.4, 1.4, 1.4, 1.5, 1.5, 1.4, 1.4, 1.4, 0 and 0 for *Eps317_1_E*) and (0 1.24 1.2 1.2 1.2 1.2 1.2 1.2 1.2 0.00 0 for Eps_317_1_L) starting from sowing to germination, emergence, end of juvenile, floral initiation, anthesis, start of grain filling end of grain filling physiological maturity, ripe and harvest respectively.

The transpiration efficiency (*transp_eff_cf units*) is 0, 0.007, 0.007, 0.007, 0.007, 0.007, 0.007, 0.007, 0, 0 and 0 for *TX9425*; 0, 0.008, 0.02, 0.005, 0.005, 0.005, 0.005, 0.008, 0, and 0 for *Franklin*; 0, 0.008, 0.008, 0.008, 0.008, 0.008, 0.008, 0.008, 0.008, 0 and 0 for *Eps_317_1_E* and 0, 0.009, 0.009, 0.009, 0.009, 0.009, 0.009, 0.009, 0.009, 0 and 0 for the *Eps_317_1_L*. starting from sowing to germination, emergence, end of juvenile, floral initiation, anthesis, start of grain filling end of grain filling physiological maturity, ripe and harvest respectively.

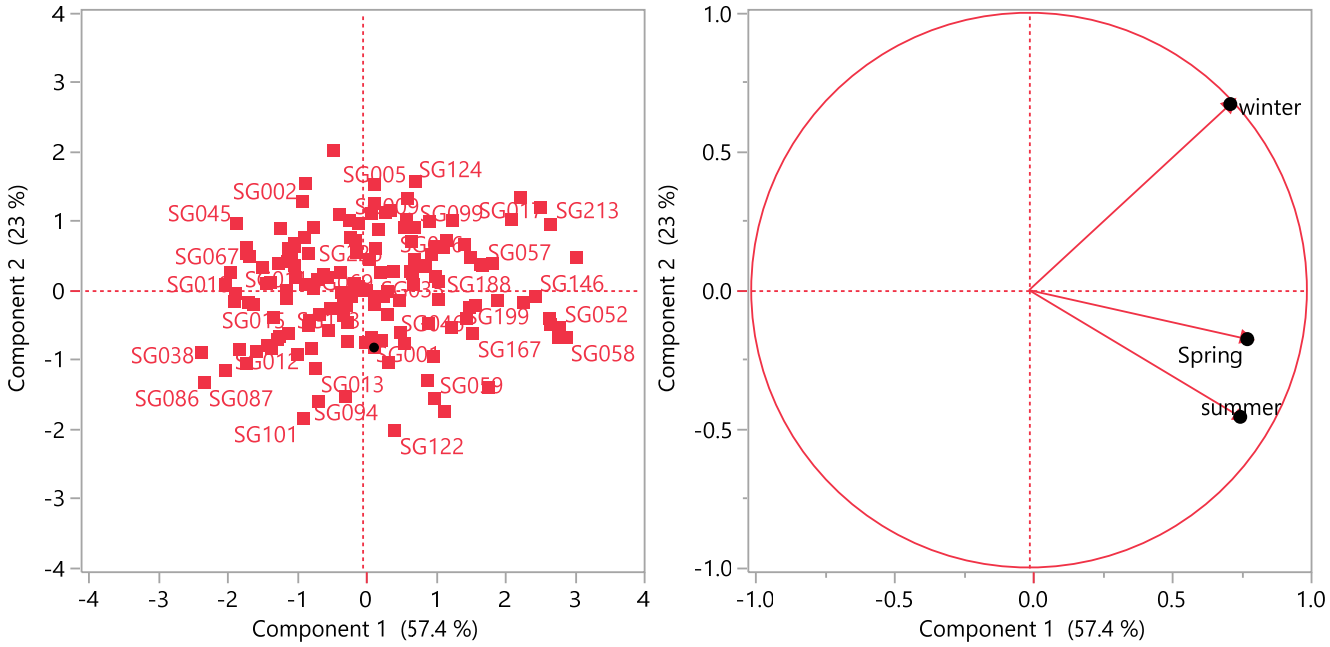

Figure 8.1 Principal components analysis (based on correlation matrix) of heading date in 173 SYR01 x Gairdner DH lines under winter, summer and spring sowing dates